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Towards a molecular understanding of titin

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Titin is at present the largest known protein (M_r 3000 kDa) and its expression is restricted to vertebrate striated muscle. Single molecules span from M- to Z-lines and therefore over 1 μm. We have isolated cDNAs encoding five distant titin A-band epitopes, extended their sequences and determined 30 kb (1000 kDa) of the primary structure of titin. Sequences near the M-line encode a kinase domain and are closely related to the C-terminus of twitchin from *Caenorhabditis elegans*. This suggests that the function of this region in the titin/twitchin family is conserved throughout the animal kingdom. All other A-band sequences consist of 100 amino acid (aa) repeats predicting immunoglobulin-C2 and fibronectin type III globular domains. These domains are arranged into highly ordered 11 domain super-repeat patterns likely to match the myosin helix repeat in the thick filament. Expressed titin fragments bind to the LMM part of myosin and C-protein. Binding strength increases with the number of domains involved, indicating a cumulative effect of multiple binding sites for myosin along the titin molecule. We conclude that A-band titin is likely to be involved in the the ordered assembly of the vertebrate thick filament.

Key words: MLCK-like kinase/titin/twitchin/vertebrate muscle

Introduction

Muscle thick filaments are complex structures consisting of several hundred protein molecules, principally myosin. In many muscle types, particularly in striated muscle, the filament is assembled with remarkable precision, probably correct to one molecule. How this process is controlled is not known, since synthetic thick filaments formed *in vitro* from purified proteins are always heterogeneous to some degree. The mechanism of regulation of thick filament assembly *in vivo* is therefore an important unsolved problem.

Titin has emerged as a candidate for a controller of filament assembly, since single molecules span from Z- to M-lines (~1 μm) in vertebrate striated muscle (Fürst *et al.*, 1988; Whiting *et al.*, 1989). The protein is expressed and laid down in striated patterns early in myogenesis (Tokuyasu and Maher, 1987; Fürst *et al.*, 1989; Isaacs *et al.*, 1989; Colley *et al.*, 1990) and partial sequences show multiple putative myosin binding motifs (Labeit *et al.*, 1990). The molecular cloning and sequencing of a significant fraction of titin and the availability of expressed and correctly folded fragments from *Escherichia coli* now allows us to explore the function of titin in filament assembly in detail.

Results

Molecular cloning of titin

Titin-specific monoclonal antibodies have been described that recognize one titin epitope each half-sarcomere (Whiting *et al.*, 1989). Pools of these antibodies were used to screen rabbit and human muscle cDNA libraries. Sequences reactive to BD6, DF12, CH11 and AB5, respectively, were isolated (Figure 1), complementing our previously published CE12 data (Labeit *et al.*, 1990). In all cases, except for CE12, several independent overlapping clones were obtained. This suggests that isolated sequences correspond to those sections of titin labelled by antibodies *in situ*. BD6 and DF12 map ~70 nm and 110 nm from the end of the thick filament, respectively. CH11 and CE12 bind in the C-protein region of the thick filament. AB5 labels a 40 nm wide region 100 nm from the M-line (Whiting *et al.*, 1989). Thus cDNAs from these A-band sections have been obtained.

Modular structure of titin

The ends of CE12 and AB5 sequences were used as starting points for cDNA walking protocols. The rabbit CE12 and the human AB5 cDNAs have been extended to 20.4 and 9.3 kb contigs, respectively, and their sequences have been determined. The two contigs each contain one open reading frame (ORF) and predict peptides of 751 and 347 kDa, respectively. The CE12 ORF encodes exclusively fibronectin type III-like (class I) and immunoglobulin-C2 like (class II, Benian *et al.*, 1989) motifs (Figure 6a) which have been found in a growing number of muscle proteins (Einheber and Fischman, 1990; Lakey *et al.*, 1990; Olson *et al.*, 1990; Price *et al.*, 1990; Shoemaker *et al.*, 1990). A total of 51 type I and 19 type II domains are found in the CE12 titin sequence. Similarly, the AB5 sequences encode 17 class I and 11 class II domains (Figure 6a). The only sequence not consisting of these repeats is a motif with homology to serine/threonine protein kinases (Figure 6b).

Super-repeats in titin

All 70 domains in the rabbit titin A-band and the 19 N-terminal domains in the AB5 contig are arranged in patterns of $(I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}I\text{-}II)_n$ to form super-repeats composed of 11 single motifs (Figure 6a). The functional importance of this super-repeat pattern is suggested by sequence self-comparison (Figure 2a). Motifs 11 domains apart are more closely related in sequence to each other than to domains of one subclass within less than a super-repeat distance. Dot-plot sequence self-comparison with high stringency therefore reveals diagonals each of 1100 residues (Figure 2b).

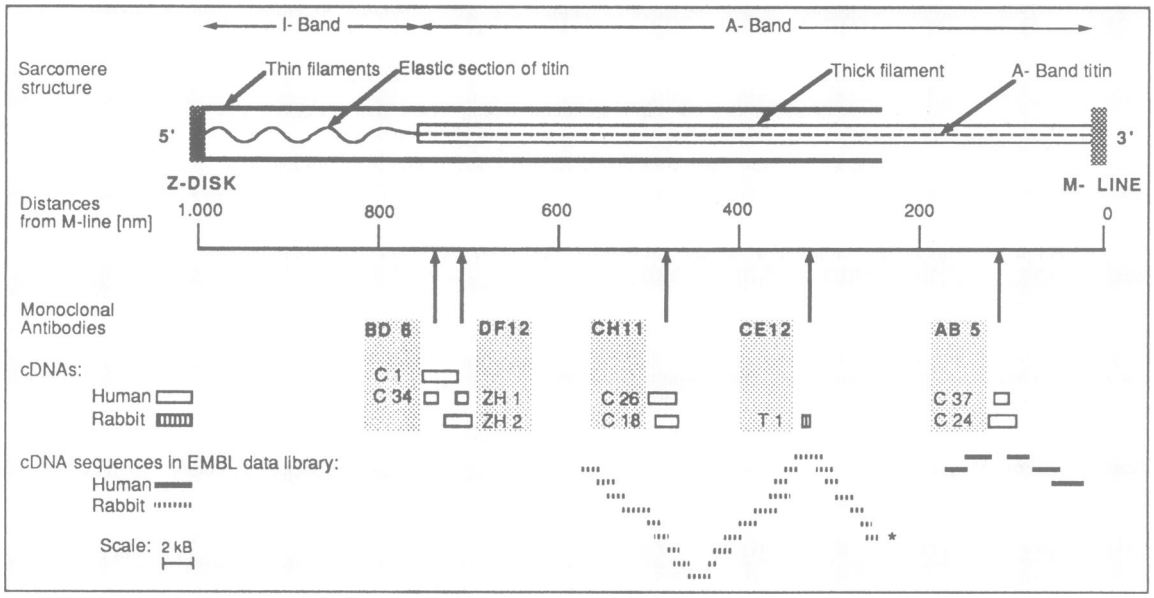

Fig. 1. Overview of the titin cloning project: relationship of titin cDNAs to the sarcomere structure and monoclonal antibody epitopes. Titin cDNAs were isolated from human heart (open boxes) and rabbit psoas (filled boxes) cDNA libraries with titin specific monoclonal antibodies and are tentatively shown as mapping in the sarcomere to where the antibodies stain. In the case of BD6, DF12, CH11 and AB5, this assumption was confirmed by independent isolates that share sequence overlap. Antibodies and corresponding cDNA clones are overlaid in grey. CE12 and AB5 clones were extended by cDNA walks, sequenced and submitted to the EMBL data library (CE12: 20 kb rabbit A-band titin, EMBL data library accession number X64696; AB5: 9.3 kb human M-line-proximal titin, EMBL data library accession number X64697). Note that the linkage of BD6/DF12 and CH11/CE12 predicts the titin orientation in the sarcomere as the 5′-end being the Z-disc region and the 3′-end the M-line.

The nine C-terminal modules of the AB5 contig are not arranged in the $(I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}I\text{-}II\text{-}I\text{-}I\text{-}I\text{-}II)_n$ pattern (Figure 6a). Therefore, titin domains near the M-line are not arranged into 1100 residue super-repeats.

Interactions with the thick filament

In order to investigate the interactions made by A-band titin with the other thick filament proteins, we assayed binding of expressed titin fragments from CH11 derived sequences to a series of muscle and non-muscle proteins. Binding was observed to myosin and C-protein, and therefore to those thick filament proteins present in the CH11 region. In the case of myosin, the binding site could be mapped by comparing titin fragment binding to the proteolytic myosin subfragments S1 and LMM (Figure 3). The observed binding patterns are compatible with a titin binding region in the LMM part of the myosin molecule.

To investigate these interactions in more detail, fragment series ranging from one to seven domains were expressed in *E.coli* and their binding characteristics assayed. We were unable to locate specific myosin binding sites in titin using the deletion series, since all tested constructs including single class I and class II motifs were found to bind (Figure 4). Comparison of the apparent binding affinities showed that binding constants increased non-linearly with titin fragment length ($Ka \approx 10^3$/M for single class I domains up to 10^7/M for the 8 domain fragment). Fragments derived from the CE12 region of the thick filament showed the same characteristics (data not shown). Consistent with the idea that most of the A-band titin sequence interacts with the other components of the thick filament, a high degree of sequence conservation between rabbit and human is observed along the complete length of a super-repeat (Figure 5).

Relationship to twitchin

Human cDNA isolates representing the AB5 epitope near the M-line were extended N- and C-terminally by cDNA walking. N-terminal extensions showed patterns of class I and class II domains consistent with 11 domain super-repeat arrangement. C-terminal walks isolated less regularly arranged domains (Figure 6a) that flank a motif with homology to serine/threonine kinases (Figure 6b). The best homology was observed to the catalytic subunits of vertebrate myosin light chain kinases (Guerriero *et al.*, 1986; Takio *et al.*, 1986; Roush *et al.*, 1988) and to the C-terminal kinase domain in twitchin (Benian *et al.*, 1989), an invertebrate giant muscle protein from *C.elegans* also consisting of class I and class II modules. The arrangement of kinase-motif flanking modules is identical in titin and twitchin.

Discussion

Electron microscopy (EM) studies of purified titin molecules have revealed a beaded structure for the molecule and it has therefore been suggested that titin consists of globular repeats over most of its length (Trinick *et al.*, 1984; Whiting *et al.*, 1989). The primary structure of titin so far determined encodes ~1000 kDa protein and therefore roughly a third of the ~3000 kDa molecule (Maruyama *et al.*, 1984; Kurzban and Wang, 1988). Since these data consist almost entirely of fibronectin- and immunoglobulin-like domains, it appears likely that the whole 3000 kDa protein is essentially composed of ~300×10 kDa globular domains. Other high molecular weight modular proteins have been recognized as important structural components of muscle (Wang and Wright, 1988; Labeit *et al.*, 1991), membranes (Koenig *et al.*, 1988; Barton *et al.*, 1988), cell adhesion and cell

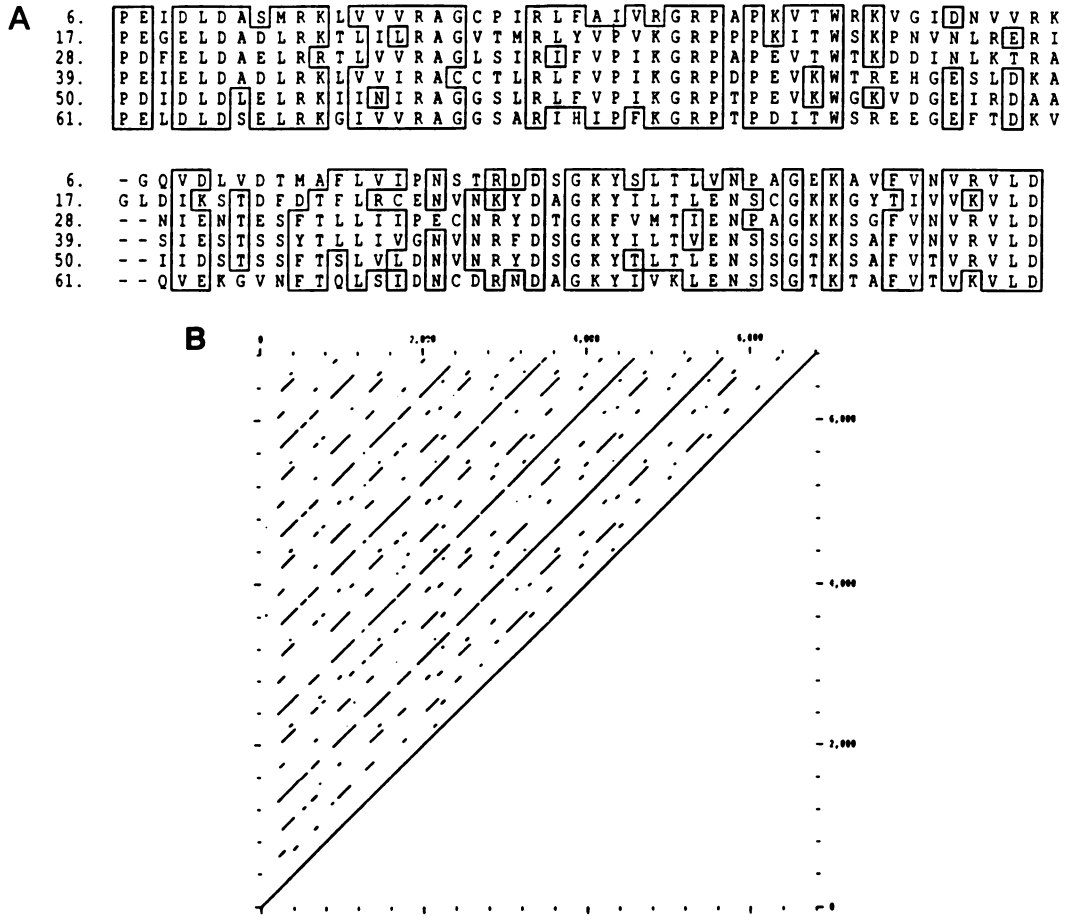

Fig. 2. The titin A-band super-repeat. (**A**) Comparison of titin A-band class II motifs from the 20 kb rabbit A-band contig. Numbers indicate positions of respective domains within the contig. Domains 1100 residues apart share 75% sequence similarity and 50% identity. This compares with the 50% similarity and 30% identity to the consensus sequence of all class II members. (**B**) Sequence self-comparison along the 20 kb rabbit titin A-band contig. Using a high stringency (60%), the single 100 aa residue repeats are suppressed and a stronger periodicity, corresponding to the 11 domain super-repeat (I-I-I-II-I-I-II-I-I-I-II), is observed every 1100 residues. The 20 kb rabbit nucleotide sequence has been submitted to the EMBL data library (accession number X64696).

recognition (Edelman, 1987). Since individual domains may diverge in their recognition specifities, modular proteins can develop complex assembly properties during evolution. In the case of vertebrate muscle, many protein molecules have to assemble accurately to form thick filaments. The data presented here suggest that a modular protein, unique in its number of domains and in the regularity of their arrangement, has developed in vertebrates to control this process.

As a consequence of this concept, the primary structure of titin should reflect the 43 nm thick filament repeat in a correspondingly repetitive pattern of attachment sites for thick filament proteins. Consistent with this idea, a strong 1100 residue periodicity (11 domains) is observed within A-band titin (Figure 2). The size of a single domain has been estimated by EM to be 4 nm (Trinick *et al.*, 1984; Whiting *et al.*, 1989) and titin-specific antibodies have been described that recognize repetitive epitopes at 43 nm intervals (Fürst *et al.*, 1989) suggesting a length of 43 nm/11 domain super-repeat. The presence of non-myosin proteins at 11 sites 43 nm apart in each half A-band has not been explained previously, since myosin heads are located one-third of this distance apart (e.g. Craig and Offer, 1976). The 1100 residue super-repeat in titin and the demonstrated interaction with C-protein show how assembly of this region of the thick filament is likely to be specified. In agreement with the idea that the 1100 residue super-repeat is involved in thick filament assembly, the super-repeat pattern terminates near the M-line (AB5 contig, Figure 6a) and near the A/I band junction (BD6 contig, data not shown). Near the M-line, the loss of the super-repeat coincides roughly with the edge of the bare zone. Possibly, this is related to the transition in myosin packing from parallel to antiparallel.

The regularity of the 11-domain super-repeat architecture of the A-band region of titin underscores the role of this giant protein not only as a connection between the thick filaments and the Z-line, but also as an important integral structural component of thick filaments. This role is indicated by the high degree of conservation between human, rabbit and mouse titin A-band sequences (Figure 5) as well as the high-affinity binding of multiple domain titin fragments to other components of the thick filament, namely the LMM portion of myosin and C-protein (Figure 3). Since the binding affinities to myosin (Figure 4) as well as to C-protein (data not shown) do not increase in a clearly logarithmic length-dependent manner, individual domains may differ in their affinities to myosin and C-protein and therefore may be specialized for interaction with particular ligands.

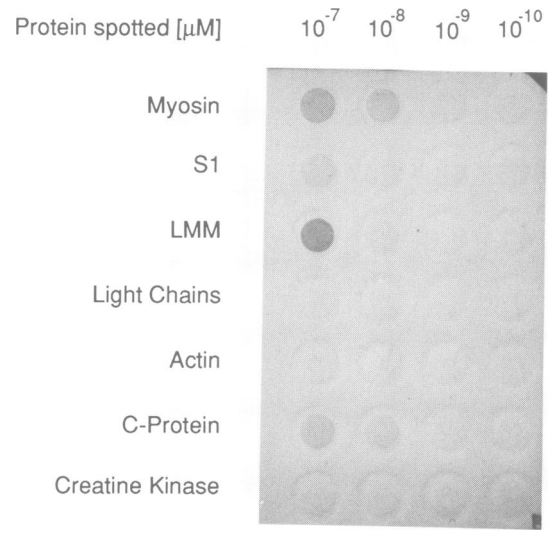

Fig. 3. Interaction of expressed A-band titin with other muscle proteins. Myosin, S1, LMM, mixed light chains, actin and C-protein were spotted onto a nitrocellulose filter, applying 10^{-13} to 10^{-16} M/spot, and reacted with an expressed, purified 8 domain titin A-band fragment at 1 nM (clone C26, Figure 1; EMBL data library accession number X64698, bp 268–2742). Bound titin fragments were detected using the monoclonal antibody CH11 (Whiting *et al.*, 1989). Binding to the rod portion of myosin and to C-protein was observed. The binding to specific regions in the rod portion of myosin reinforces the idea that titin interacts with myosin to control thick filament assembly.

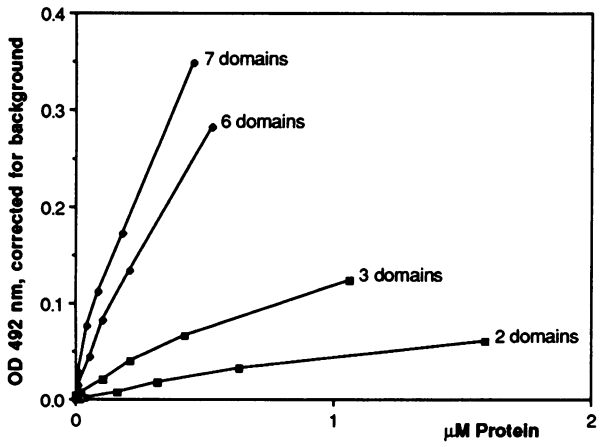

Fig. 4. Titin binding to myosin is cumulative along its length. A deletion series ranging from two to seven domains of the human clone C26 (Figure 1) was expressed, and purified products were reacted at various concentrations with myosin covalently coated to microtitre plates. Values are corrected for a BSA background. Binding increased with fragment length, indicating a cumulative effect of multiple myosin binding sites in the multi-domain fragments. Estimated values for K_a derived from the initial slopes of the binding curves show a non-linear behaviour of ln K_a, indicating varying affinities to myosin of the different domains (data not shown).

Immunological methods have shown a wide range of titin related high molecular weight proteins in invertebrates (Nave and Weber, 1990; Lakey *et al.*, 1990; Nave *et al.*, 1991). For two members of this class, twitchin and projectin, sequences have shown them to be composed of domain patterns with the simpler repeat $(I\text{-}I\text{-}II)_n$ (Benian *et al.*, 1989; Ayme-Southgate *et al.*, 1991). In the case of twitchin, the complete primary sequence has been determined and

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1
TLKWAPPKDDGGSEITNYILEKRDSVNNKWVTCASAVQKTTFRVTRLHEGMEYTFRVS
   G
AENKYGVGEGLKSEPIVARHPFDVPDAPPPPNIVDVRHDSVSLTWTDPKKTGGSPITG
                 K                              R
YHLEFKERNSLLWKRANKTPIRMRDFKVTGLTEGLEYEFRVMAINLAGVGKPSLPSEP
  I              K
VVALDPIDPPGKPEVINITRNSVTLIWTEPKYDGGHKLTGYIVEKRDLPSKSWMKANH
               V                                        T
VNVPECAFTVTDLVEGGKYEFRIRAKNTAGAISAPSESTETIICKDEYEAPTIVLDPT
I  D                                  G
IKDGLTIKAGDTIVLNAISILGKPLPKSSWSKAGKDIRPSDITQITSTPTSSMLTIKY
                                                        V
ATRKDAGEYTITATNPFGTKVEHVKVTVLDVPGPPGPVEISNVSAEKATLTWTPPLED
 S                 e   R          I
GGSPIKSYILEKRETSRLLWTVVSEDIQSCRHVATKLIQGNEYIFRVSAVNHYGKGEP
       V                A         V         L
VQSEPVKMVDRFGPPGPPEKPEVSNVTKNTATVSWKRPVDDGGSEITGYHVERREKKS
                      G                    T          y       G
LRWVRAIKTPVSDLRCKVTGLQEGSTYEFRVSAENRAGIGPPSEASDSVLMKDAAYPP
     T                N                        D  NY      V  A
GPPSNPHVTDTTKKSASLAWGKPHIDGGLEITGYVVEHQKVGDEAWIKDTTGTALRIT
    AR                y                        T V     p
QFVVPDLQTKEKYNFRISAINDAGVGEPAVIPDVEIVEREMAPDFELDAELRRTLVVR
E     H
AGLSIRIFVPIKGRPAPEVTWTKDNINLKNRANIENTESFTLLIIPECNRYDTGKFVM
                      D   t
TIENPAGKKSGFVNVRVLDTARPSPQLRPTDITKDSVTLHWDLPLIDGGSRITNYIVE
                   Pg vln
KREATRKSYSTATTKCHKCTYKVTGLSEGCEYFFRVMAENEYGIGEPTETTEPVKASE
        V                                              S  k
APSPPDSLNIMDITKSTVSLAWPKPKHDGGSKITGYVIEAQRKGSDQWTHITTVKGLE

CVVRNLTEGEEYTFQVMAVNSAGRSAPRESRPVIVKEQTMLPELDLRGIYQKLVIAKA

GDNIKVEIPVLGRPKPTVTWKKGDQILKQTQRVNFETTATSTILNINECVRSDSGPYP
                      V          V N          S
LTARNIVGEVGDVITIQVHDIPGPPTGPIKFDEVSSDFVTFSWDPPENDGGVPISN
  K                                          E
                                                        1100
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Fig. 5. Conservation of titin A-band sequences between rabbit and human. One complete human titin A-band super-repeat protein sequence (**top**) is compared with the corresponding sequence in rabbit (**bottom**, only sequence differences are shown). On the protein level, a sequence similarity of 99% and identity of 95% is seen along the complete length of the super-repeat. The lesser degree of similarity on the nucleotide level (93%) indicates that the majority of exchanges are silent or conservative (capital letters are for conservative, small letters for non-conserved exchanges). The 3.3 kb human titin sequence derives from the CH11 isolates C18 and C26 (Figure 1) and has been submitted to the EMBL data library (accession number X64698). The rabbit sequence was obtained by 5′ walks starting from the CE12 epitope and is part of the 20 kb rabbit titin contig (accession number X64696). A similar degree of homology between rabbit, human and mouse was observed in the CE12 region of the sarcomere (accession numbers X64696, X64699 and X64700).

shown to consist at its C-terminus of less regularly arranged class I and II domains that flank a kinase domain. AB5 titin sequences near the M-line are strikingly homologous to this region in twitchin (Figure 6). Both the complex motif arrangement and the kinase domain sequences are conserved. This degree of homology between human and *C.elegans* argues for crucial conserved functions. Since muscle contraction is regulated in different ways in vertebrates and nematodes, and since kinase assays have so far failed to detect myosin light chain kinase activity of titin, it appears unlikely that the titin kinase domain is involved in the regulation of muscle contraction. It will be of interest to elucidate the function of the kinase domains in the titin/twitchin family and to identify their substrates.

The conservation of the kinase domain and flanking structures further signifies a common evolutionary ancestor for titin and twitchin (Labeit *et al.*, 1990) and justifies the consideration of twitchin as a mini-titin (Nave and Weber, 1990). C- and N-terminally of the kinase domain, both molecules diverge from their putative ancestor. C-terminally, titin must have acquired additional sequences, since cDNA walks covering > 1000 residues 3′ of the kinase domain have failed to reach the titin C-terminus. N-terminally, many

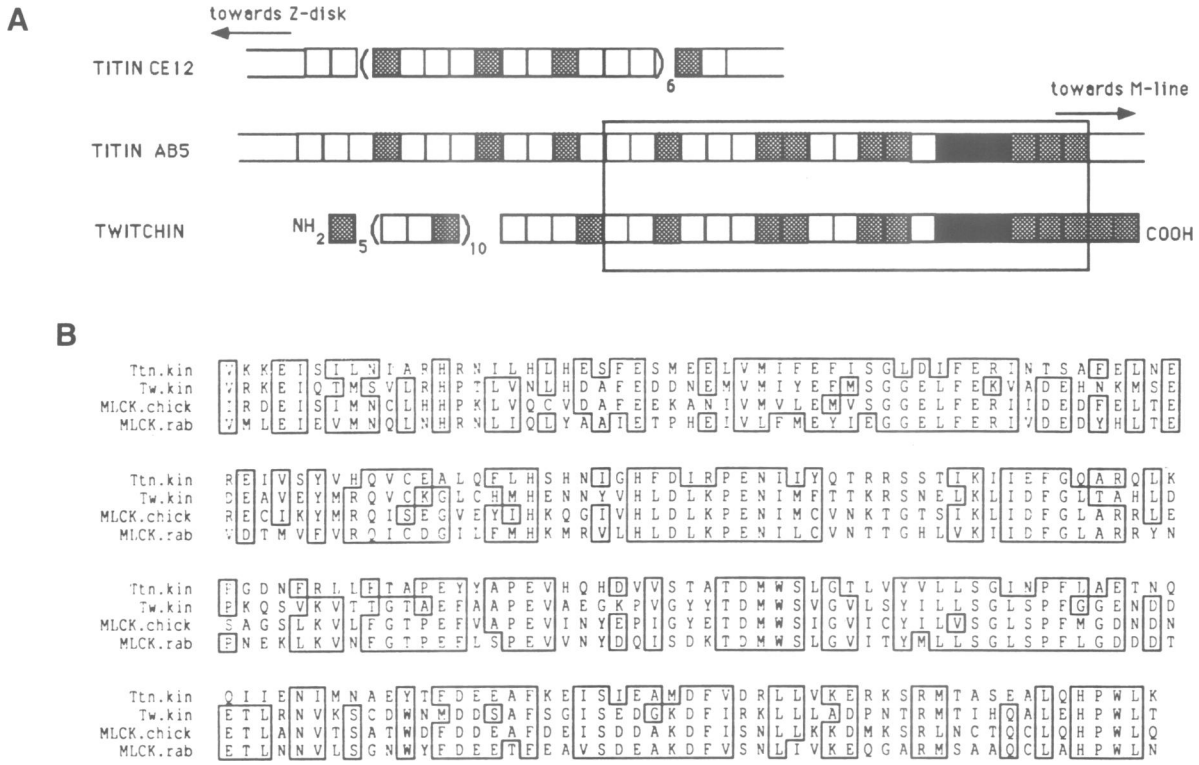

Fig. 6. Comparison of titin and twitchin. (**A**) Motif patterns in titin and twitchin. Class I motifs, open boxes; class II motifs, filled; kinase domain, black. In twitchin, the regular (I-I-II) pattern from the central region of the molecule is followed C-terminally by a more complex pattern that flanks the MLCK-like motif (adapted from Benian *et al.*, 1989). Titin within the thick filament is organized into 11 domain super-repeats (CH11 and CE12). Titin sequences near the M-line isolated with the antibody AB5 share with twitchin a region of identical organization of kinase domain and flanking modules (framed). In contrast to twitchin, titin extends much further N-terminally. Similarly, C-terminal walks covering ~1000 residues have failed to reach the C-terminus. (**B**) Comparison of the kinase motif in human titin (Ttn.kin) with the catalytic kinase domains of chicken (Olson *et al.*, 1990) and rabbit (Einheber and Fischman, 1990) myosin light chain kinases (MLCK.chick and MLCK.rab) and twitchin (Benian *et al.*, 1989). Residues conserved ≥75% are boxed. The 9.3 kb AB5 human titin nucleotide sequence has been submitted to the EMBL data library (accession number X64697).

additional domains appear to have developed during evolution, allowing the molecule to span to the Z-line. The regularity and biochemical properties of A-band modules suggest that within this region of the molecule these domains are specialized to organize thick filament assembly. It remains to be seen what function can be assigned to the three domain pattern, $(I\text{-}I\text{-}II)_n$, in twitchin and projectin. In titin, it will be important to determine how local titin structure and function relate to local sarcomere structure.

Materials and methods

Molecular cloning and sequencing of titin

Monoclonal antibodies were used to identify immunopositive titin cDNAs in λZAP rabbit psoas (S.L.) and human heart cDNA libraries (Stratagene) essentially as described (Nierendorf *et al.*, 1987), and their sequences were determined (Sanger *et al.*, 1977). For extension of rabbit cDNAs, rabbit psoas mRNA was reverse transcribed (AMV RT Life Sciences), poly(G) tagged with terminal transferase (Stratagene), and 21 RACE extensions were performed (Frohmann *et al.*, 1988) adding 14 kb to our previously published data (Labeit *et al.*, 1990). RACE and sequencing primers were used to perform analytical PCR reactions (Saiki *et al.*, 1985) with total rabbit psoas cDNA to confirm the assembled contig, and one 22 domain 3′ jumping PCR was detected (see Figure 1). For human walks, end-specific probes randomly labelled with ^{32}P (Feinberg and Vogelstein, 1983) were used to isolate clones from the human heart library. Primary positives were subjected to anchored PCR (Rasmussen *et al.*, 1989) to isolate overlapping fragments with 1−2 kb 3′ and 5′ extensions. Amplified fragments were subcloned into M13 (Yanisch-Perron *et al.*, 1985) and their sequence determined (Sanger *et al.*, 1977). From the obtained sequences, new sets of end-specific primers were used to repeat the walking procedure and five walks yielding 7.5 kb were obtained.

Sequence analysis and interpretation

Sequence editing, fragment assembly, prediction of ORFs and dotplot self-comparison were performed with the UWGCG software package (Devereux *et al.*, 1984). The single predicted ORF in the human AB5 contig was used to translate the encoded peptide, and the C-terminal segments not consisting of class I or II modules were used to search the Swiss Prot data library (release 19) with Wordsearch (UWGCG). Significant homologies were found to serine/threonine kinases and the best matches were to MLCKs and twitchin. Motifs were aligned by eye and conserved positions were boxed with Prettyplot (P.Rice, EMBL).

Protein expression and purification

Titin sequences ranging from one to eight domain fragments were isolated by PCR (Saiki *et al.*, 1985) using the CE12 and CH11 cDNA isolates as templates (EMBL data library AC X64698 bp 268−2742). Obtained fragments were subcloned into the pET8c vector (Studier *et al.*, 1990) and fused N-terminally with an oligonucleotide linker encoding a His_6 tag sequence. After induction of BL21 cells (Studier and Moffat, 1991) with 0.5 mM IPTG for 3 h, the harvested cell pellet was sonicated in 50 mM sodium phosphate pH 8.0, 500 mM NaCl, 0.2% Tween 20. After centrifugation at 25 000 *g*, soluble expressed products from the supernatant were purified by metal chelate affinity chromatography on Ni^{2+}−NTA agarose (Qiagen) essentially as described (LeGrice and Grueninger-Leitch, 1990). Aliquots of purified products were assayed by circular dichroism, and results showed predominantly the presence of β-sheet structures.

Solid phase titin binding assays

For dot-blot assays, purified expressed products were reacted in binding buffer (1% BSA, 200 mM KCl, 20 mM sodium phosphate pH 7.0, 0.2% Tween 20) at a concentration of 1 nM titin fragment with muscle proteins dotted at various concentrations onto nitrocellulose previously blocked with 0.1% fish skin gelatin (Sigma) in binding buffer. Titin fragment bound to nitrocellulose was detected using the monoclonal titin antibody CH11 (Whiting *et al.*, 1989). Bound antibody was visualized after reaction with

anti-mouse-alkaline phosphatase conjugate (Sigma) and the color substrates 5-bromo-4-chloro-3-indolylphosphate and nitrotetrazolium blue. For quantification of the binding of titin fragment to myosin, purified titin fragments were reacted to myosin covalently coated to Covalink plates (Nunc) using the bifunctional cross-linker disuccinimidylsuberate essentially as described (Staros *et al.*, 1986). For titin/myosin binding, expressed titin fragments in binding buffer were added to plates blocked with 1% bovine serum albumin and 0.1% fish skin gelatin. After 10 h incubation at 20°C, unbound protein was washed off and bound protein detected in an ELISA as described (Bugari *et al.*, 1990) using the monoclonal antibody CH11 (Whiting *et al.*, 1989). Bound antibody was detected using anti-mouse-peroxidase conjugate and 1,4-diaminobenzene as colour substrate. Extinctions at 492 nm were measured using a titertec multiscan ELISA plate reader. Control plates were coated with bovine serum albumin alone.

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References

Ayme-Southgate,A., Vigoreaux,J., Benian,G. and Pardue,M.L. (1991) *Proc. Natl. Acad. Sci. USA*, **88**, 7973–7977.

Barton,C.H., Dickson,G., Gower,H.J., Rowett,L.H., Putt,W., Elsom,V., Moore,S.E., Goridis,C. and Walsh,F.S. (1988) *Development*, **104**, 165–73.

Benian,G.M., Kiff,J.E., Neckelmann,N., Moerman,D.G. and Waterston,R.H. (1989) *Nature*, **342**, 45–50.

Bugari,G., Poirsi,C., Beretta,G., Ghielmi,S. and Albertin,A. (1990) *Clin. Chem.*, **36**, 47–52.

Colley,N.J., Tokuyasu,K.T. and Singer,S.J. (1990) *J. Cell Sci.*, **95**, 11–22.

Craig,R. and Offer,G. (1976) *Proc. Roy. Soc. Lond. B.*, **192**, 451–461.

Devereux,J., Haeberli,P. and Smithies,O. (1984) *Nucleic Acids Res.*, **12**, 387–395.

Edelman,G.M. (1987) *Immunol. Rev.*, **100**, 11–45.

Einheber,S. and Fischman,D.A. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2157–2161.

Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **13**, 6–13.

Frohmann,M.A., Dush,M.K. and Martin,G.R. (1988) *Proc. Natl. Acad. Sci. USA*, **85**, 8998–9002.

Fürst, D.O., Osborn, M., Nave,R. and Weber,K. (1988) *J. Cell Biol.*, **106**, 1563–1572.

Fürst,D.O., Osborn,M. and Weber,K. (1989) *J. Cell Biol.*, **109**, 517–527.

Fürst,D.O., Nave,R., Osborn,M. and Weber,K. (1989) *J. Cell Sci.*, **94**, 119–125.

Guerriero,v.J.R., Russo,M.A., Olson,N.J., Putkey,J.A. and Means,A.R. (1986) *Biochemistry*, **25**, 8372–8381.

Isaacs,W.B., Kim,I.S., Struve,A. and Fulton,A.B. (1989) *J. Cell Biol.*, **109**, 2189–2195.

Koenig,M., Monaco,A.P. and Kunkel,L.M. (1988) *Cell*, **53**, 219–26.

Kurzban,G.P. and Wang,K. (1988) *Biochem. Biophys. Res. Commun.*, **155**, 1155–1161.

Labeit,S., Barlow,D.P., Gautel,M., Gibson,T., Hsieh,C.-L., Francke,U., Leonard,K., Wardale,J., Whiting,A. and Trinick,J. (1990) *Nature*, **345**, 273–276.

Labeit,S., Gibson,T., Lakey,A., Leonard,K., Zeviani,M., Knight,P., Wardale,J. and Trinick,J. (1991) *FEBS Lett.*, **282**, 313–316.

Lakey,A., Ferguson,C., Labeit,S., Reedy,M., Larkins,A., Butcher,G., Leonard,K. and Bullard,B. (1990) *EMBO J.*, **9**, 3459–3467.

LeGrice,S.F.J. and Grueninger-Leitch,F. (1990) *Eur. J. Biochem.*, **187**, 3007–3014.

Maruyama,K., Kimura,S., Yoshodomi,H., Sawada,H. and Kikuchi,K. (1984) *J. Biochem. Tokyo*, **95**, 1423–1433.

Nave,R. and Weber,K.J. (1990) *J. Cell Sci.*, **95**, 535–544.

Nave,R., Fürst,D., Vinkemeier,U. and Weber,K. (1991) *J. Cell Sci.*, **98**, 491–496.

Nierendorf,R.C., Percy,C. and Young,R.A. (1987) *Methods Enzymol.*, **152**, 458–469.

Olson,N.J., Pearson,R.B., Needleman,D.S., Hurwitz,M.Y., Kemp,B.E. and Means,A.R. (1990) *Proc. Natl. Acad. Sci. USA*, **87**, 2284–2288.

Price,M.G., Brooks,C.A. and Gomer,R.H. (1990) *J. Cell Biol.*, **111**, 170a.

Rasmussen,U.B., Basset,P. and Daniel,J.-Y. (1989) *Nucleic Acids Res.*, **17**, 3308.

Roush,C.L., Kennelly,P.J., Glaccum,M.B., Helfman,D.M., Scott,J.D. and Krebs,E.G. (1988) *J. Biol. Chem.*, **263**, 10510–10516.

Saiki,R.K., Scharf,S.J., Faloona,F., Mullis,G.T. and Erlich,H.A. (1985) *Science*, **230**, 1350–1354.

Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463–5467.

Shoemaker,M.O., Lau,W., Shattuck,R.L., Kwiatkowski,A.P., Matrisian,P.E., Guerra-Santos,L., Wilson,E., Lukas,T.J., van Eldik,L.J. and Watterson,D.M. (1990) *J. Cell Biol.*, **111**, 1107–1125.

Staros,V.V., Wright,R.W. and Swingle,D.M. (1986) *Anal. Biochem.*, **156**, 220–222.

Studier,F.W. and Moffat,B.A. (1991) *J. Mol. Biol.*, **189**, 113–130.

Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1990) *Methods Enzymol.*, **185**, 62–89.

Takio,K., Blumenthal,D.K., Walsh,K.A. and Krebs,E.G. (1986) *Biochemistry*, **25**, 8049–8057.

Tokuyasu,K.T. and Maher,P.A. (1987) *J. Cell Biol.*, **105**, 2781–2801.

Trinick,J., Knight,P. and Whiting,A. (1984) *J. Mol. Biol.*, **180**, 331–356.

Vallee,R.B. (1991) (ed.), *Molecular Motors and the Cytoskeleton. Methods in Enzymology*, Vol. 196.

Wang,K. and Wright,J. (1988) *J. Cell Biol.*, **107**, 2199–2212.

Whiting,A., Wardale,J. and Trinick,J. (1989) *J. Mol. Biol.*, **205**, 163–169.

Yanisch-Perron,C., Vieira,J. and Messing,J. (1985) *Gene*, **33**, 103–119.

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