Repetitive titin epitopes with a 42 nm spacing coincide in relative position with known A band striations also identified by major myosin-associated proteins

An immunoelectron-microscopical study on myofibrils

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Summary

A direct titin–thick filament interaction in certain regions of the A band is suggested by results using four new monoclonal antibodies specific for titin in immunoelectron microscopy. Antibodies T30, T31 and T32 identify quasi-repeats in the titin molecule characterized by a 42–43 nm repeat spacing. These stripes seem to coincide with striations established by others on negatively stained cryosections of the A band. Antibodies T30 and T32 recognize epitopes matching five or two of the seven striations per half sacromere known to harbor both the myosin-associated C-protein and an 86K ($K = 10^3 M_r$) protein. Antibody T31 labels two stripes in the P zone, which correspond to the two positions where decoration is seen with 86K protein, but not with C-protein. The single titin epitope defined by antibody T33 is located 55 nm prior to the center of the M band. This position seems to coincide with the M7 striation defined by others on negatively stained A bands. The T33 epitope position proves that the titin molecule, which is known to be anchored at the Z line, also penetrates into the complex architecture of the M band. The titin epitopes described here enable us to begin to correlate known ultrastructural aspects of the interior part of the A band with the disposition of the titin molecule in the sarcomere. They raise the question of whether there is a regular interaction pattern between titin and the thick filaments. Because of the limit of resolution of immunoelectron microscopy, it remains to be seen whether the repetitive titin epitopes of the C and P zones coincide exactly with the position of the myosin-associated proteins or lie on a similar lattice, out of register with these proteins.

Key words: elastic filament, C-protein, myosin, skeletal muscle, titin.

Introduction

Sarcomeric muscles have a highly ordered structure. This involves not only the well-known thick and thin filaments with their associated proteins, but also an elastic component, which is thought to be present as a third filament system (for recent reviews, see Maruyama, 1986; Wang, 1985). Given the relative abundance of titin in isolated myofibrils of skeletal and heart muscle, this giant protein is likely to be a major component of the third filament system. Although nebulin, another high molecular weight protein of skeletal muscle myofibrils, has also been proposed as a component of elastic filaments, recent experiments in several laboratories show that it is not present in cardiac muscle (Hu *et al.* 1986; Locker & Wild, 1986; Hoffman *et al.* 1987; Fürst *et al.* 1988; Wang & Wright, 1988) and so is unlikely to be a general elastic component of all sarcomeric muscles.

In low-porosity polyacrylamide gels, dodecyl sulfate-solubilized myofibrils show a typical titin doublet, designated TI and TII. Only TII, thought to be a proteolytic derivative of the larger TI species (Maruyama, 1986; Wang, 1985), can be extracted under native conditions (Kimura & Maruyama, 1983; Trinick *et al.* 1984; Wang *et al.* 1984). Although electron micrographs of purified TII show marked heterogeneity, the molecules appear as very thin and rather long strings (Maruyama *et al.* 1984; Trinick *et al.* 1984; Wang *et al.* 1984). Length estimates for titin molecules range from ~0·6 to 1·2 μm and so a single titin molecule could span the distance from the M band to either the N_2 line (Wang, 1985) or the Z line (Maruyama, 1986). Strong experimental support for titin binding to the Z line comes from immunoelectron micrographs using 10 distinct titin monoclonal antibodies, each defining a unique epitope along the half sarcomere. The polar epitope map starts at the Z line and

ends 0·2 μm before the center of the M band (Fürst *et al.* 1988). Antibodies decorating at or close to the Z line are specific for the larger TI component, whereas all other antibodies react with both components of the titin doublet. Thus the titin filament seems attached *via* the TI-specific end to the Z line structure. Since only epitopes located between the N_1 line and the A/I junction show stretch dependency in location, the putative elastic part of the titin filaments may be restricted to this region.

Here we report on a second group of titin monoclonal antibodies, which begin to define possible interaction patterns of titin with the A band. In immunoelectron microscopy, antibodies T30, T31 and T32 define multiple quasi-repeats of the titin molecule spaced 42–43 nm apart. These occur in the region of the A band close to the M band. The positions of these new repetitive titin epitopes seem to coincide with seven of the nine stripes previously reported for some myosin-associated proteins, i.e. C-protein and 86K ($K = 10^3 M_r$) protein (Craig & Offer, 1976; Bähler *et al.* 1985*a*,*b*). We discuss some structural implications of this observation.

Materials and methods

Monoclonal antibodies

The new monoclonals T30 to T33 arose in a fusion of PAI cells with spleen cells of a Balb/c mouse previously immunized with purified TII (cf. Fürst *et al.* 1988). Because, initially, immunofluorescence microscopy pointed to A band decoration, these antibodies were not considered important, as several A band-specific epitopes had already been documented by immunoelectron microscopy (Fürst *et al.* 1988). Later these hybridomas were made monoclonal by subcloning *via* limiting dilution and further characterized by immunofluorescence microscopy, Western blotting and immunoelectron microscopy. It became clear that they represented a new group. T30–T33 were studied as hybridoma supernatants. In addition, T30 and T33 were available as antibodies purified from ascites fluids elicited in Balb/c mice primed with pristane. Rabbit antibodies to C-protein and to 86K protein from chicken breast muscle have been described (Bähler *et al.* 1985*a*,*b*). They were generously provided by Drs H. Eppenberger and T. Wallimann, ETH, Zürich, Switzerland. Titin antibody T23 was previously characterized by immunoelectron microscopy (Fürst *et al.* 1988).

Gel electrophoresis and immunoblotting

Linear polyacrylamide gradient gels (2% to 10% acrylamide and 0·5% crosslinker) were used without a stacking gel in the Laemmli (1970) buffer system as described (Fürst *et al.* 1988). Polypeptides were electrophoretically transferred to nitrocellulose. Antibody decoration was revealed with peroxidase-labeled rabbit anti-mouse antibodies (Dakopatts, Copenhagen, Denmark) followed by peroxidase substrates. Immunofluorescence microscopy on frozen sections and isolated myofibrils was as described (Fürst *et al.* 1988).

Immunoprecipitation

Affinity-purified sheep anti-mouse antibodies were coupled to CNBr-activated Sepharose 4B (Pharmacia). They were used to absorb murine antibody T33 from overgrown supernatant. The Sepharose-bound antibody sandwich was used to absorb titin TII from a crude high-salt extract of myofibrils, which had been dialyzed against buffer T (50 mM-Tris–HCl, pH 7·9, 80 mM-KCl, 2 mM-EGTA, 1 mM-2-mercaptoethanol, 1 mM-NaN_3). After thorough washing three times with buffer T, twice with buffer T plus 0·5 M-KCl, and twice with phosphate-buffered saline, the beads were harvested and incubated in hot SDS sample buffer for 15 min at 50°C. Supernatants were subjected to electrophoresis.

Immunoelectron microscopy

Observations were made on fiber bundles and isolated myofibrils from chicken breast muscle. Tissue preparation and antibody labeling were performed after a modification of the method of Dennis *et al.* (1984) as described earlier (Fürst *et al.* 1988). After skinning of muscle strips with Triton X-100, antibody decoration for 12 h at 4°C was done with either 5- to 10-fold concentrated (Centricon 10 concentration cells, Amicon) hybridoma supernatants or antibodies purified from ascites fluid (final concentration ~0·5 mg ml^{-1}). After several washes, muscle strips were treated with antigen-affinity-purified sheep anti-mouse antibody (0·3 mg ml^{-1}) and washed again as described. Muscles used as controls were first treated with hybridoma growth medium and then with sheep anti-mouse antibody. Specimens were fixed with 1% glutaraldehyde in solution B (10 mM-phosphate buffer, pH 7·0, 100 mM-KCl, 5 mM-$MgCl_2$, 1 mM-EGTA, 1% glucose, 1 mM-iodoacetamide, 0·1 mM-phenylmethylsulfonyl fluoride (PMSF), 0·02% NaN_3) for 30 min on ice, washed in 0·1 M-Na cacodylate buffer, pH 7·0, three times for 10 min, postfixed in 1% OsO_4 in Na cacodylate buffer, dehydrated in a graded series of ethanol and embedded in Epon. Ultrathin sections were positively stained with uranyl acetate and Reynold's lead citrate.

In addition, antibodies against C-protein and 86K protein were purified by ammonium sulfate fractionation of the serum and used at final IgG concentrations of around 1 mg ml^{-1}. Sheep anti-rabbit antibodies, purified on rabbit IgGs, were employed as second antibody (final concentration 0·5 mg ml^{-1}).

Myofibrils to be used for negative staining were prepared essentially as described by Knight & Trinick (1982). These were treated only with first antibodies and negatively stained with 1% aqueous uranyl acetate.

Results

Characterization of titin antibodies

The monoclonal antibodies T30–T33 were isolated after a fusion of mouse PAI myeloma cells with spleen cells of a Balb/c mouse immunized with native titin II (Materials and methods). Hybridomas were made monoclonal by limiting dilution. Western blots of whole extracts of chicken breast muscle showed that T30, T31 and T32 reacted specifically with both bands of the titin doublet (TI and TII in the nomenclature of Wang *et al.* 1984) and revealed no reaction with any other myofibrillar polypeptide (Fig. 1). Antibody T33 did not detect titin in blotting experiments. The titin specificity of this IgM antibody (Table 1) could be determined by immunoprecipitation using a crude high-salt extract of myofibrils of chicken breast muscle (Fig. 1). All antibodies were further characterized as to immunoglobulin type and cross-reactivity patterns using immunofluorescence microscopy on frozen sections of various sarcomeric muscles. Table 1 shows the broad cross-species reactivity of the four antibodies among vertebrates.

Immunoelectron microscopy

Fiber bundles of chicken pectoralis major muscle were extracted with Triton X-100 and incubated with the titin monoclonal antibodies. Sheep anti-mouse immunoglobulins were used to amplify the signal. Samples were fixed with glutaraldehyde, postfixed with osmium and embedded in plastic. Ultrathin longitudinal sections were examined by electron microscopy (Fig. 2). Antibody T33 provided a decoration pattern similar to those observed earlier with 10 distinct titin antibodies (Fürst *et al.* 1988), i.e. only one decoration line occurred per half sarcomere (Fig. 2D; see also Fig. 4). The epitope of T33 is located at the edge of the M band, 55 nm away from the center of this structure. The T33 epitope marks the closest known apposition between the titin molecule and the M band revealed by the currently available titin antibody banks (Fürst *et al.* 1988; Whiting *et al.* 1989) (see Fig. 4, below).

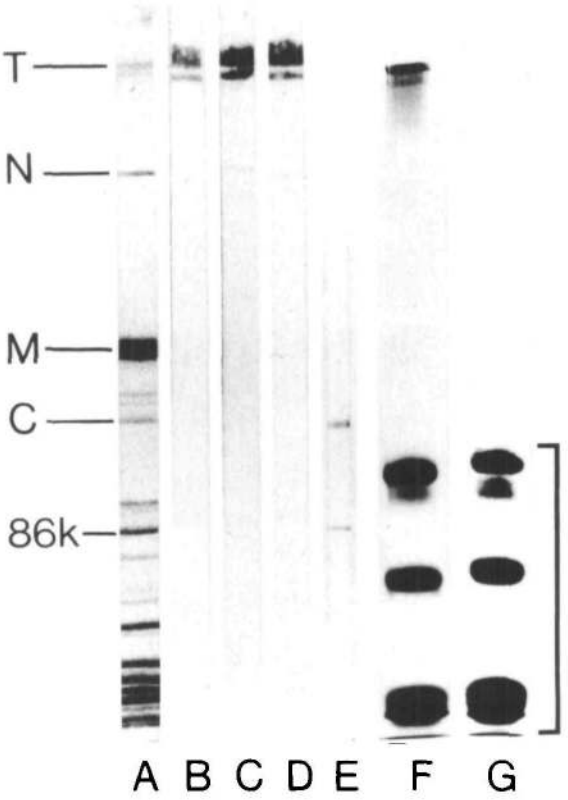

Fig. 1. Antibody specificity by immunoblotting and immunoprecipitation. Lane A, Ponceau Red-stained blot of whole extract of chicken skeletal muscle. The positions of the titin doublet (T), nebulin (N), myosin heavy chain (M), C-protein (C), and 86K protein (86K) are indicated. Lanes B–D are immunoblots with antibodies T30, T31 and T32, respectively. Lane E is an immunoblot with a mixture of rabbit antibodies against C-protein and 86K protein. Lane F is the result of an immunoprecipitation using antibody T33 on a high-salt muscle extract to isolate titin TII (for details see Materials and methods). Lane G is the corresponding control showing only the immunoglobulin bands (indicated by bracket).

Table 1. *Properties of monoclonal antibodies*

Clone number:	T30	T31	T32	T33
Immunoglobulin type:	IgG1	IgG1	IgG1	IgM
Fish skeletal muscle (*Torpedo* sp.)	+	+	+	+
Salamander skeletal muscle (*Ambystoma tigrina*)	+	+	–	+
Chicken skeletal muscle	+	+	+	+
Chicken heart muscle	+	+	+	+
Mouse skeletal muscle	+	+	+	+
Pig skeletal muscle	+	+	+	+
Human skeletal muscle	+	+	+	–

Summary of the reaction of the monoclonal antibodies with various muscles, assayed by immunofluorescence microscopy of frozen sections.

Antibodies T30, T31 and T32 defined a new subgroup of our titin antibodies, since they exhibited multiple decoration lines within the half sarcomere. Although all lines occurred within the A band, their number and relative position were dependent on the specific antibody used. Thus antibody T30 provided five lines situated at 268, 310, 353, 395 and 438 nm from the center of the M band. The decoration lines at 268 and 395 nm were always very prominent, whereas the other three lines seemed weaker (Fig. 2A). Antibody T31 provided two decoration lines, situated 140 and 183 nm from the center of the M band (Fig. 2B). Antibody T32 decorated at two positions per half sarcomere, i.e. at 353 and 395 nm from the center of the M band (Fig. 2C).

Further experiments confirmed that, within the resolution of immunoelectron microscopy, the positions of the two T32 epitopes correspond to two of the five positions labeled by the T30 antibody. When antibodies T30 and T32 were mixed, immunoelectron microscopy showed five decoration lines per half sarcomere (Fig. 2E). Their position reflected the sum of the T30 and T32 epitopes from the single antibody experiments (for a better orientation see the summary in Fig. 4). In a further control experiment (Fig. 2F) antibodies T30 and T23 were mixed. T23 has been previously shown to detect a single epitope located ~200 nm from the M band center (Fürst *et al.* 1988). Five of the six decoration lines obtained per half sarcomere, i.e. the ones corresponding to T30, showed the 42–43 nm repeat pattern. The sixth line (T23 epitope) was about 65 nm from the last T30 epitope. This confirmed that the position of the T23 epitope does not lie on the 42–43 nm repeat of the P and C zones (for nomenclature of P and C zones, see Sjöström & Squire, 1977).

Antibodies T30–T32 identify repetitive titin epitopes, which in all cases occur at a regular spacing of 42–43 nm over a limited range for the A band (between 140 nm and 440 nm from the M band center) (see also Fig. 4). Similar spacings along the interior part of the A band have been reported by Craig & Offer (1976) and Bähler *et al.* (1985*b*) for certain myosin-associated proteins such as the C-protein and the 86K protein. These authors detected a total of nine stripes. Seven of these stripes (225, 268, 310, 353, 395, 438 and 480 nm) are revealed by antibodies to C-protein and to 86K protein, whereas the two stripes situated more closely to the M band (140 and 183 nm) are defined only by antibodies to the 86K protein (for a summary see Fig. 4). In order to relate the repetitive

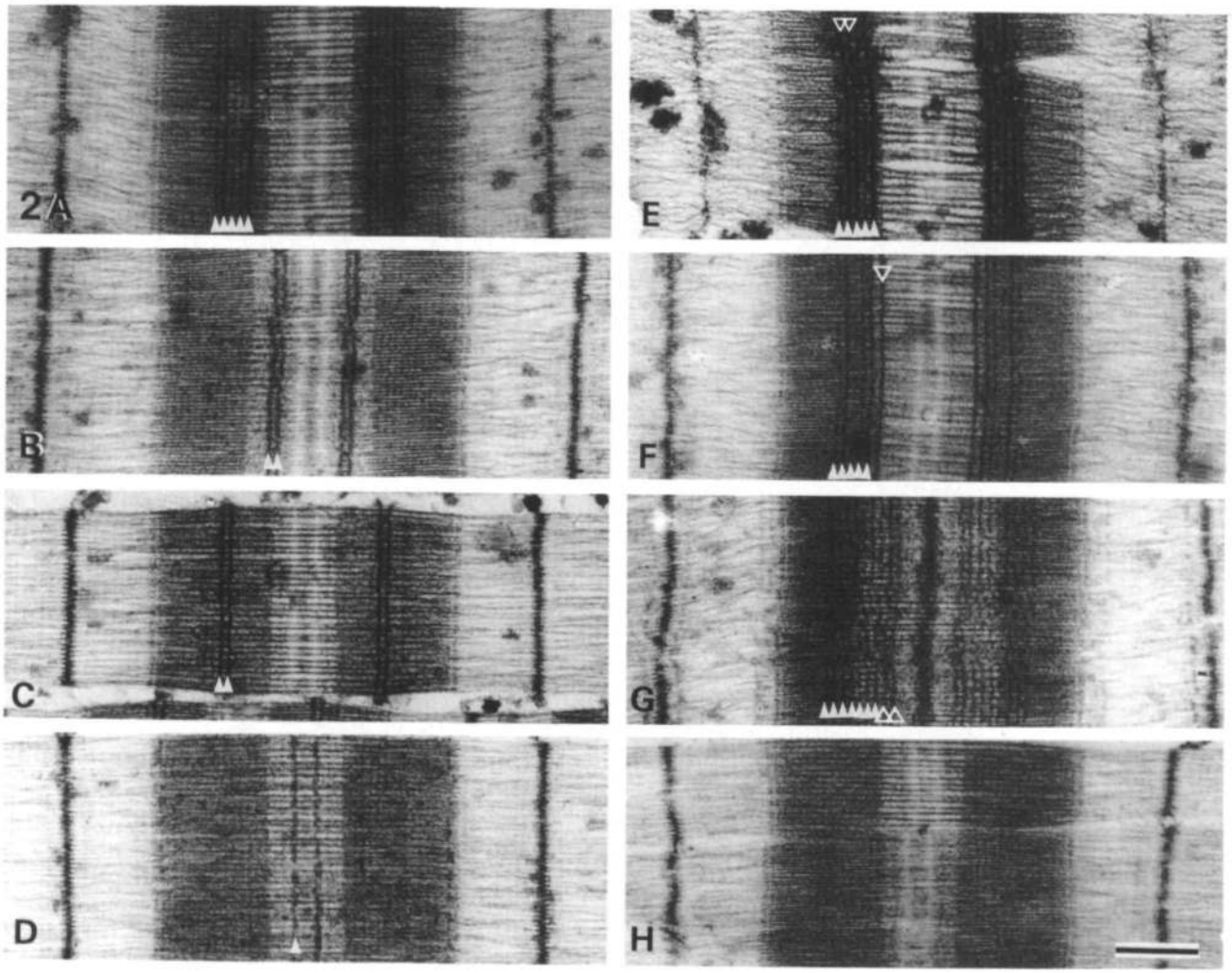

Fig. 2. Immunoelectron microscopical localization of titin epitopes in chicken pectoralis major muscle with different monoclonal antibodies using the procedure outlined in Materials and methods. Antibodies used are: T30 (A), T31 (B), T32 (C) and T33 (D). The labeling positions are indicated in one half sarcomere with arrowheads. The following mixtures of antibodies were used as controls: E, T30 (arrowheads) plus T32 (open triangles); F, T30 (arrowheads) plus T23 (open triangle); G, rabbit antibodies against C-protein (arrowheads) and 86K protein (open triangles plus arrowheads). Note that the label of T32 coincides in its position with two of the epitopes recognized by T30 (E). On the other hand, the label of T23 does not occur in the 42–43 nm repeat revealed by T30 (F). H is a control muscle treated only with second antibody. Bar, 500 nm.

epitopes documented by our titin antibodies T30–T32 to the stripes defined by the two myosin-associated proteins, we repeated the immunoelectron microscopical analysis under our experimental conditions. Fig. 2G shows, for example, the nine decoration lines provided by a mixture of rabbit antibodies to C-protein and to 86K protein. Labeling of isolated myofibrils followed by negative staining provided a direct correlation of the observed decoration patterns with the documented striations in the P and C zones. The mixture of T30 and T32 enhanced five of the seven strong lines in the C zone (Fig. 3A; for comparison see also Fig. 2E). A further control was done using the mixture of C-protein antibodies and T30. This yielded decoration of all seven lines in the C zone without significant broadening of the stripes in comparison to single antibody experiments. Thus within the limits of resolution of immunoelectron microscopy the observed repeat patterns of T30 and C-protein seem to coincide in their position (Fig. 3B). Owing to the thickness of the specimen this method did not yield the same level of resolution as the ultrathin sections of plastic-embedded muscle. Therefore we preferred the latter method in general.

The combined results show a striking coincidence of the titin epitopes and the positions of C-protein and the 86K protein (Fig. 4). Although none of our titin antibodies covered the first stripe defined by the myosin-associated proteins, stripes 3 and 4 were covered by T32, and T30 bound to stripes 2–6. No titin counterpart has yet been found for the seventh stripe (the last C-protein stripe) but the two remaining stripes, which are defined by 86K antibodies, were also detected by T31. Fig. 4 provides a summary of the established map of titin epitopes along the sarcomere.

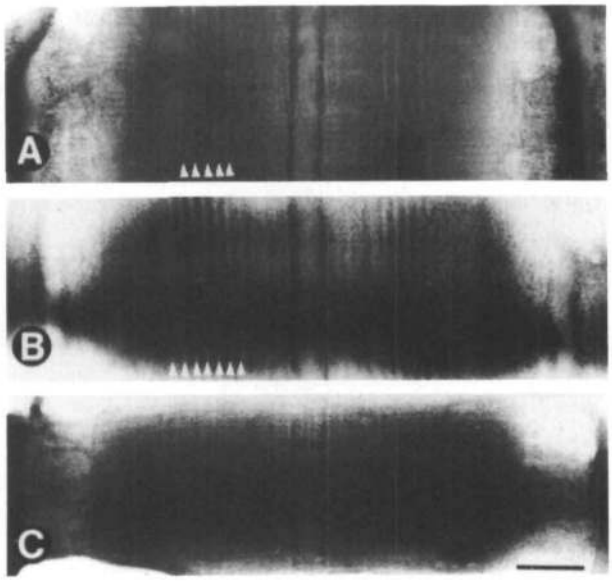

Fig. 3. Negatively stained isolated myofibrils previously labeled with a mixture of monoclonal titin antibodies T30 plus T32 (A) or with a mixture of T30 and rabbit antibody against C-protein (B). Labelling positions are marked with arrowheads. Note that the epitope positions of T30 and T32 overlap and they lie on the 42–43 nm repeat that is revealed by C-protein antibodies. C is a control myofibril without antibody. Bar, 250 nm.

Discussion

The new titin epitopes described by T30–T33 can be directly correlated with the current view of the ultrastructure of the A band. Negatively stained ultra-thin cryosections of isolated A-segments show a number of prominent stripes, which appear at regular distances along the A band (Craig, 1977; Hanson *et al.* 1971; Sjöström & Squire, 1977). A striking feature of such micrographs is the complexity of the M band in the middle of the A band. Sjöström & Squire (1977) described a central M1 line and up to eight additional lines (M2 to M9) within the preceding 70 nm of each half sarcomere. Although the exact role of these lines is not yet known, it has been suggested that some of them are associated with the location of specific M band proteins. Muscle creatine kinase is restricted to M1 and M4 (Wallimann *et al.* 1975; Strehler *et al.* 1983), while the 165K M-protein seems to occur in M6 and in the ensheathments around the thick filaments (Masaki & Takaiti, 1974; Strehler *et al.* 1983; Wallimann *et al.* 1983; see also reviews by Wallimann & Eppenberger, 1985; and Squire *et al.* 1987). Myomesin, a 185K protein of the M band is found exclusively in the ensheathments (Grove *et al.* 1984). Within this conceptual framework the single titin epitope recognized by antibody T33 is very interesting. In immunoelectron micrographs T33 provides a single decoration line per half sarcomere. As this line is located at 55 nm from M1 (Figs 2, 4) it seems to coincide with the M7 line, one of the weaker striations in the M band defined by Sjöström & Squire (1977). Thus in the M-region titin may contribute to a cross-linking of neighboring thick filaments and participate in the earlier reported structural organizations of the center of the A band.

Prominent transverse striations of the A band occur also outside the M band. According to Sjöström & Squire (1977) there are seven prominent stripes in the 'C zone' and two stripes in the 'P zone'. The appearance of these nine stripes, which occur between 225 nm and 480 nm from M1 and show a 42–43 nm spacing, has been connected with two prominent myosin-associated proteins. A 140K polypeptide, called C-protein, occurs in the seven stripes of the C zone (Craig & Offer, 1976), while the 86K protein is found in the same stripes and also in the two stripes of the P zone (Bähler *et al.* 1985*a*,*b*). T30, T31 and T32 are the only monoclonal titin antibodies in our bank that, as shown by immunoelectron microscopy, define repetitive epitopes. As seen in Figs 2 and 3, these show a pronounced 42–43 nm spacing (for other repetitive epitopes see below). The decoration lines provided by T30 and T32 seem to coincide with five of the seven stripes postulated for the C zone, while T31 marks the position of the two stripes in the P zone. Although in antibody mixing experiments we did not observe any band broadening (Figs 2 and 3), the limit of resolution of the immunoelectron microscopical methods is about 10 nm for the band width. Thus, at present we cannot decide between two possibilities: either the 42–43 nm spacing of repetitive epitopes coincides with the stripes provided by the myosin-associated proteins, or it is located on a similar lattice slightly out of register with the stripes.

We have previously argued that titin molecules connect Z and M lines and thus integrate I/Z/I brushes with A bands. A linear epitope map of ten non-repetitive epitopes starting at the Z line with a TI-specific epitope extended to 200 nm from the center of the M band (Fürst *et al.* 1988). This map can now be extended into the M band. The T33 epitope has been located at a position equivalent to the M7 striation seen in negatively stained cryosections. It occurs 55 nm from the center of the M band. This location provides further support for the involvement of titin in sarcomere organization. The map of more than ten A band-specific epitopes so far assembled (Fig. 4) rules out the hypothesis that titin filaments form an interior core of thick filaments (Locker, 1984). Instead it emphasizes the interpretation deduced from isolated thick filaments after negative staining (Trinick *et al.* 1984). These authors noticed fine 'string-like structures' connected to the ends of the thick filaments and located parallel to the filaments over long distances. Putative titin strings were observed approximately 50 nm from the axis of the thick filaments, i.e. 10 nm beyond the location of myosin heads. Our epitope mapping results document multiple points of possible connections between the titin filament and the A band, by immunelectron microscopy. Antibody T3 marks the tip of isolated native thick filaments (Hill & Weber, 1986) and labels the A/I junction of decorated myofibrils (Fürst *et al.* 1988). The seven epitopes described by antibodies T30–T32 coincide within the limits of resolution with documented striations displayed by A bands not subjected to antibody decoration. Thus it seems also

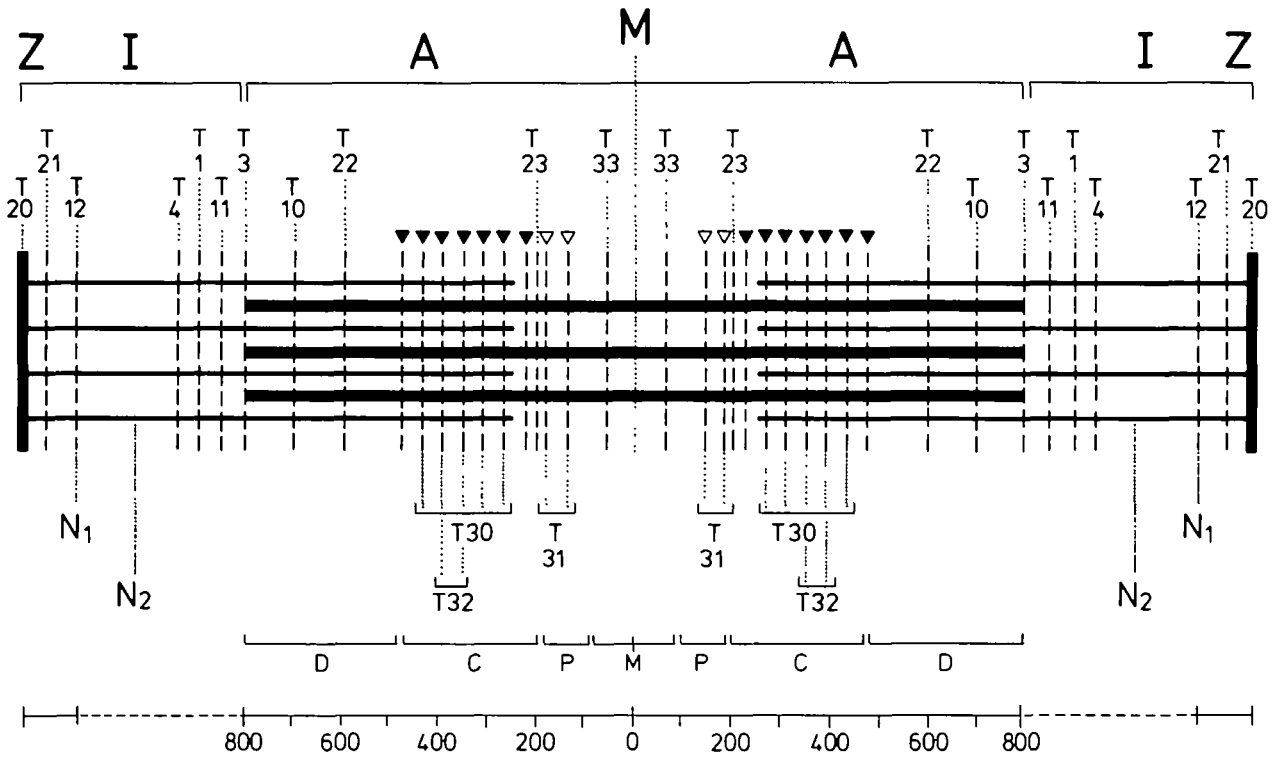

Fig. 4. Summary of the myofibrillar decoration patterns obtained by immunoelectron microscopy with 14 monoclonal antibodies to titin. Z, I and A mark the known band positions and M identifies the center of the M line. Approximate positions of the N_1 and N_2 lines are also shown. Unique titin epitopes are indicated above the sarcomere. They are identified by the previously described 11 monoclonal antibodies (T1, T2, T3, T4, T10, T11, T12, T20, T21, T22 and T23) (Fürst *et al.* 1988) and by T33 (see Results). Note that these antibodies label only a pair of transverse lines symmetrical to the M band. The T33 epitope lies within the M band proper, about 55 nm from the center of the M band (see also Fig. 2). Its position seems to coincide with the M7 line of the M band as defined by Sjöström and Squire, (1977). T30–T32 are the first monoclonal antibodies in our bank, which identify repetitive epitopes. Their position is indicated below the sarcomere. These repetitive epitopes are spaced at 42–43 nm. They seem to correspond to some of the nine regularly spaced striations in the C and P zone of the A band (Sjöström and Squire, 1977), which are marked by triangles. Filled triangles mark the seven stripes containing C-protein and 86K protein (C stripes), open symbols indicate the two additional stripes (P stripes), which harbor only the 86K protein (Bähler *et al.* 1985*a*,*b*). Note that the three titin antibodies T30–T32 together cover seven of the nine striations connected to the two myosin-associated proteins. T31 detects both P stripes, while T30 detects five of the seven C stripes. The scale at the bottom reveals the distances within the A band in nm. The broken line indicates the elastic portion of the titin molecule.

that in the interior part of the A band there is an intimate relation between titin and myosin filaments. Whether this disposition occurs directly or *via* a now specified set of myosin-associated proteins remains to be determined. Prime candidates are the C-protein and the 86K protein. A very interesting study in this context is a three-dimensional reconstruction of thick filaments from frog skeletal muscle by Stewart & Kensler (1986), who suggested that accessory proteins such as C-protein are arranged as a ring encircling the thick filament shaft at 43-nm intervals. Thus myosin-associated proteins could bridge the gap between the titin string and the thick filament. Since Moos *et al.* (1978) have reported binding of C-protein to light meromyosin and myosin subfragment-2, it will be important to see whether this myosin-associated protein can also bind to titin.

Repetitive titin epitopes seem also to occur outside the P and C zones of the A band as seen in two reports, which appeared after the completion of our analysis. Itoh *et al.* (1988) described two monoclonal antibodies. Antibody SM1 labeled two stripes in the I band prior to the A/I junction, with the exact location and spacing influenced by the contractional state of the muscle. Antibody 3B9 yielded three stripes: the A/I junction and positions 50 and 150 nm within the A band. In a similar region, Whiting *et al.* (1989) have also detected repetitive epitopes. Their antibody CF5 labeled the following three positions: 80 nm within the A band, the A/I junction, and approximately 40 nm within the I band. It may be more than a coincidence that the value of the epitope spacing is again around 42 nm, as established in detail for the P and C zones (see above). The presence of repetitive epitopes, so far documented in each case only for a limited array of the titin filament, raises the question of repetitive structural units. This seems to be a molecular principle, since large filamentous proteins and their subunits such as, for instance, spectrin and dystrophin are built from domains containing multiple quasi-repeats (Speicher *et al.* 1983; Koenig *et al.* 1988). A similar repeat pattern can therefore also be expected for the very

much longer titin molecule. Given the documentation of many unique epitopes along the titin molecule (Fürst *et al.* 1988; Whiting *et al.* 1989; see also Fig. 4), such repeating units cannot be fully identical. However, the titin molecule could well be built from consecutive quasi-repeats. Whether these reflect the 4 nm beads seen in some rare electron micrographs (Trinick *et al.* 1984; Wang *et al.* 1984) will require the future characterization of titin by cDNA-derived sequences.

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Note added in proof: We have meanwhile made use of antibodies T30 and T32 to visualize the polarity of isolated titin molecules by immunoelectron microscopy (R.N., D.O.F. and K.W., *J. Cell Biol.*, in press).