Nuclear Titin Interacts with Histones

Lan King, PhD; Chun-Ru Jhou, MSc

Background: Titin is a giant muscle protein that is responsible for the elasticity of muscle and may also function as a molecular scaffold for myofibrillar assembly. However, its presence in the nucleus remains elusive.

Methods: Nuclear titin was extracted with 0.15 M sodium phosphate buffer containing 0.3 M NaCl, followed by ammonium sulfate fractionation and gel filtration column chromatography. The proteins not separable from titin were subjected to mass spectrometry (MS) and MS/MS analyses.

Results: This purification procedure removed almost all impurities except three low molecular weight proteins (12-15 kDa). These proteins were not separated from titin through various types of column chromatography, indicating their strong interaction with nuclear titin. These proteins were identified as histones H2A, H3 and H4 by MS and MS/MS analyses.

Conclusion: The existence of titin in the nucleus was confirmed by obtaining purified titin from the nucleus. Furthermore, nuclear titin exhibited strong interaction with histones H2A, H3 and H4, suggesting its role as a chromosome scaffold.
(Chang Gung Med J 2010;33:201-10)

Key words: titin, nucleus, histone, purification, protein interaction, proteomics

Chromosomes are highly ordered, elastic structures that maintain their integrity throughout the physically strenuous process of cell division. Chromosomal DNA must be compacted to fit inside the cell nucleus and further compacted 5-10 fold during mitosis. To fully understand the mitotic chromosome structure, it is necessary to identify the protein components of the chromosome and to learn the role each protein plays in chromosomal architecture. Several proteins required for chromosome condensation have been identified, including histones, topoisomerase II and the structural maintenance of chromosomes (SMCs). The SMCs are comprised of the condensin and cohesion complexes, which mediate chromatin condensation and sister chromatid cohesion, respectively.[1-4]

Titin, the largest known polypeptide chain, is a giant elastic muscle protein with a molecular weight of 3000-3700 kDa.[5,6] Vertebrate titins are highly modular, and are comprised mostly of immunoglobulin (Ig)-like and fibronectin type 3 (Fn3)-like domains arranged in tandem.[7] Titin also contains an elastic region rich in proline (P), glutamic acid (E), valine (V), and lysine (K) residues, called the PVEK domain,[8] and a carboxy-terminal serine kinase domain.[9] Titin is responsible for the elasticity of muscle and also functions as a molecular scaffold specifying the correct assembly of myofibrils.[10,11] Machado *et al* reported that chromosomes contain a titin-like molecule. They isolated the gene that encodes the drosophila homologue of vertebrate titin (D-titin). The D-titin antibodies stain condensed

From the Department of Biochemistry and Molecular Biology, Collage of Medicine, Chang Gung University, Taoyuan, Taiwan.
Received: Apr. 28, 2009; Accepted: Jun. 4, 2009
Correspondence to: Prof. Lan King, Department of Biochemistry and Molecular Biology, Chang Gung University, 259, Wunhua 1st Rd., Gueishan Township, Taoyuan County 333, Taiwan (R.O.C.) Tel.: 886-3-2118800 ext. 5159; Fax: 886-3-2118452; E-mail: lanking@mail.cgu.edu.tw

human and drosophila mitotic chromosomes,[12] as well as insect spermatocyte mitotic spindles.[13] Mutations in D-titin cause chromosome undercondensation, chromosome breakage, loss of diploidy and premature sister chromatid separation.[14] Zastrow *et al*. showed that the C-terminus of nuclear titin binds lamins in vivo and might contribute to nuclear organization during interphase.[15] Qi *et al*. identified the nuclear location of titin's amino-terminal region and proposed a role for it in promoting cell division.[16]

A model for chromosome structure has been proposed based on elasticity measurements, specifically the longitudinal deformability and bending rigidity of whole chromosomes. This model proposes that the condensed chromosomes are formed by one or several thin rigid elastic axes built of titin-like molecules, surrounded by a soft envelope of chromatin.[3] However, Takata *et al*. performed titin array analyses and found no titin transcript in HeLa cells. They also analyzed HeLa cell extract by gradient gel and found no bands in the ultra-high molecular weight region. Based on these results at both the transcript and protein levels, they concluded that titin with a molecular weight higher than 1000 kDa is absent in metaphase chromosomes as well as in HeLa cells.[17]

In this study, we showed that titin with a molecular weight similar to its counterpart in muscles is present in the nucleus. Furthermore, our results indicate that nuclear titin interacts strongly with histones H2A, H3 and H4.

METHODS

Nucleus isolation

To prevent oxidation and degradation of titin catalyzed by endogenous proteases, all solutions for nucleus preparation and titin purification included 1 mM DTT, 1 μM trans-epoxysuccinyl-L-leucylamido(4-guanidino)-butane (E-64), 1 μM pepstatin and 1 mM phenylmethylsulfonyl fluoride (PMSF). Nucleus preparation was performed according to published procedures with slight modification.[18,19] Briefly, rabbit uterus dissected free of adipose tissue was washed with phosphate buffered saline (PBS) and cut into small pieces. The minced tissue was homogenized with 15 volumes of 10 mM Tris-HCl buffer containing 0.25 M sucrose and 10 mM $MgCl_2$, pH 7.5 in a blender with six 10-sec bursts and 10-sec intervals and filtered through two layers of surgical gauze to remove connective tissue, followed by centrifugation at 5000 rpm (JA-17 rotor, Beckman, Palo Alto, CA, U.S.A.) for 12 min. The pellet was resuspended in 15 volumes of 10 mM Tris-HCl buffer containing 2.2 M sucrose and 10 mM $MgCl_2$, pH 7.5, followed by blending for three 10-sec bursts and 10-sec intervals. The mixture was centrifuged at 30,000 rpm (Type 35 rotor, Beckman, Palo Alto, CA, U.S.A.) for 1 hour.

Isolation and purification of nuclear titin

The pellet of nuclei was suspended in 5 volumes of 50 mM sodium phosphate buffer containing 10 mM ethylenediamine tetra-acetic acid (EDTA) and homogenized in a blender with seven 10-sec bursts and 10-sec intervals. The homogenate was centrifuged at 14,000 rpm for 15 min using a JA-17 rotor. The pellet was homogenized and centrifuged in 5 volumes of the buffer described above. The pellet from the centrifugation was thoroughly suspended in 5 volumes of 0.15 M sodium phosphate buffer containing 0.3 M NaCl, 20 mM EDTA, 10 mM DTT, 2 mM PMSF, 2 μM pepstatin, and 2 μM E-64 (Extraction Buffer) and homogenized in a blender with seven 10-sec bursts and 10-sec intervals. The extraction proceeded for two hours with gentle stirring and was centrifuged at 15,000 rpm (JA-17 rotor) for 25 min. The supernatant was called 2 h Extract. The pellet was thoroughly suspended in 4 volumes of Extraction Buffer and the extraction proceeded for approximately 20 h (overnight) accompanied by gentle stirring. The supernatant from the centrifugation was called Overnight Extract.

The extract was precipitated with 30% ammonium sulfate and centrifuged in a JA-17 rotor (Beckman) at 15000 rpm for 25 min. The supernatant was dialyzed against high-ionic-strength buffer (0.6 M NaCl, 50 mM sodium phosphate, 1 mM DTT and 1 mM EDTA, pH 7) to remove ammonium sulfate and was concentrated with 35% polyethylene glycol (35000 MW for synthesis, Merck, Hohenbrunn, Germany) in the same buffer. The concentrated sample (called 30% S) was dialyzed against high-ionic-strength buffer to remove contaminated polyethylene glycol and was applied to a Sephacryl S-500 (GE Healthcare Bio-Sciences, Uppsala, Sweden) gel filtration column (100 x 1.5

cm). The column was equilibrated and eluted with high-ionic-strength buffer at a flow rate of 0.75 ml/min and fractions were collected every 4 min. Eluted fractions were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and SDS-gradient gel electrophoresis.

SDS-gradient gel electrophoresis

Protein samples were analyzed by 2-12% SDS-gradient gel electrophoresis using a SE 250 mini-verticle gel electrophoresis unit (GE Healthcare Bio-Sciences) and a Hoefer (San Francisco, CA, U.S.A.) SG 50 gradient maker. A plunger was inserted into the mixing chamber of the gradient maker to hold the mixing volume constant and to generate exponential gradients. The volume used for 2% acrylamide is three times that used for 12% acrylamide. This gel system, called 2-12% (3:1) gradient gel, is appropriate for separating proteins with molecular weights greater than 1000 kDa. The upper region of this gel is wider than the lower region after staining.

Gel samples were heated at 60°C for at least 8 min with 50 mM DTT and 1% SDS to completely reduce intermolecular disulfide bonds.[20,21] This step is critical because titin is sensitive to oxidation and titin oligomers formed through intermolecular disulfide bonds remain in gel wells. Urea was added to dissolve the sample thoroughly if necessary. The gel sample was mixed with glycerol (final concentration greater than 10%) and bromophenol blue before loading. High molecular weight standard was synthesized by cross-linking myosin molecules into even-number myosin oligomers by p-NN"-phenylenebismaleimide.[22,23]

Western blotting

Titin samples were separated with SDS-agarose (1.5%) gel electrophoresis and transferred to a nitrocellulose membrane. After 1 h blocking with 5% milk, the nitrocellulose membrane was subsequently incubated with monoclonal anti-titin (T11, Sigma) and alkaline phosphatase-conjugated anti-mouse IgG (H+L) (Promega, Madison, WI, U.S.A.). The protein bands were visualized by incubating with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Mass spectrometric analysis

Coomassie Blue stained protein bands were excised and in-gel digested with trypsin.[24] Briefly, the gels were destained by 50 mM NH_4HCO_3 / 100% acetonitrile (3:2). The proteins were reduced with 25 mM NH_4HCO_3 containing 10 mM DTT at 56°C for 45 min, alkylated with 55 mM iodoacetamide at room temperature for 30 min, and digested overnight with trypsin (20 μg/ml) at 37°C. The tryptic peptides were acidified with 0.5% trichloroacetic acid and loaded onto a target plate (MTP AnchorChip™ 600/384 TF, Bruker-Daltonik, Bremen, Germany). Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) MS and MS/MS analyses were performed in an Utraflex MALDI-TOF mass spectrometer (Bruker-Daltonik). Monoisotopic peptide masses were assigned and used for data base search with the Mascot search engine (Matrix Science, London, U.K.).

RESULTS

The gel patterns of the samples obtained from the various purification steps of nuclear titin are shown in Fig. 1. The early washing procedures with 50 mM phosphate buffer removed proteins of molecular weights less than 1000 kDa with a slight extraction of titin (lanes 2 and 3). Titin was extracted with 0.15 M phosphate buffer containing 0.3 M NaCl for 2 hours. The 2 h Extract contained an appreciable amount of 500 kDa protein in addition to titin (lane 4). Overnight Extract contained a relatively lower amount of impurities (lane 7).

Both the 2 h Extract and Overnight Extract were subjected to 30% ammonium sulfate precipitation. The centrifugation pellet of the 30% ammonium sulfate precipitate of the 2 h Extract (2 h 30% P) contained mainly impurities (lane 5); the supernatant (2 h 30% S) contained mainly titin (lane 6). The 2 h 30% S fraction was subjected to gel filtration column chromatography. However, the eluted titin fraction contained an appreciable amount of impurities (date not shown). At least one more cycle of column chromatography was needed to collect titin of reasonable purity.

The centrifugation pellet of the 30% ammonium sulfate precipitate of the Overnight Extract (o/n 30% P) also contained mainly impurities (lane 8). The amount of impurities in the supernatant (o/n 30% S, lane 9) was much lower than that of the 2 h 30% S (lane 6). The o/n 30% S was also subjected to gel fil-

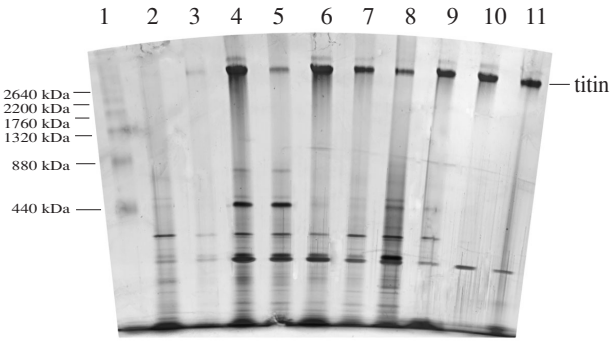

Fig. 1 Purification procedures for nuclear titin. The amount of protein loaded onto each lane is indicated in parentheses below. 1, myosin oligomers with molecular weights of 440, 880, 1320, 1760, 2200 and 2640 kDa; 2, the supernatant from centrifugation of the first washing cycle of the nuclear homogenate (0.4 μg); 3, the supernatant from centrifugation of the second washing cycle of the nuclear homogenate (0.2 μg); 4, 2 h Extract (2.9 μg); 5, 2 h 30% P (1.9 μg); 6, 2 h 30% S (2.4 μg); 7, o/n Extract (1.4 μg); 8, o/n 30% P (2.0 μg); 9, o/n 30% S (1.4 μg); 10 and 11, eluted titin fractions from the Saphacryl S-500 gel filtration column (1.0 μg). Abbreviations used: 2 h 30% P: 30% ammonium sulfate precipitate of 2 h Extract; 2 h 30% S: concentrated supernatant from 30% ammonium sulfate precipitation of the 2 h Extract; o/n Extract: Overnight Extract; o/n 30% P: 30% ammonium sulfate precipitate of the o/n Extract; o/n 30% S: concentrated supernatant from 30% ammonium sulfate precipitation of the o/n Extract.

tration column chromatography. The eluted titin fractions contained a small amount of impurities (lanes 10 and 11). One more cycle of column chromatography was usually performed to achieve higher purity. Because proteins with molecular weights less than 20 kDa moved close to the gel front in the 2-12% (3:1) gradient gel system, we performed a 12% SDS polyacrylamide gel electrophoresis (PAGE) to identify the low molecular weight impurities. Titin fractions from the column chromotography identified by both 2-12% (3:1) gradient gel and 12% SDS-PAGE are shown in Fig. 2. The titin band on the gel of Fig. 2A was further confirmed by its cross-reactivity with the monoclonal antibody against skeletal muscle titin (Fig. 2C). Three bands corresponding to molecular weights between 12 kDa and 15 kDa were detected in the titin fractions of 12% SDS-PAGE (Fig. 2B). More cycles of gel filtration column chromatography failed to separate these proteins from titin. Furthermore, various types of columns were used to separate these proteins from titin with no success. The inseparable feature between these low-molecular-weight proteins and titin suggests strong interactions among them. The molecular weights of these bands were estimated to be 12 kDa, 14 kDa, and 15 kDa. These protein bands were excised, digested in-

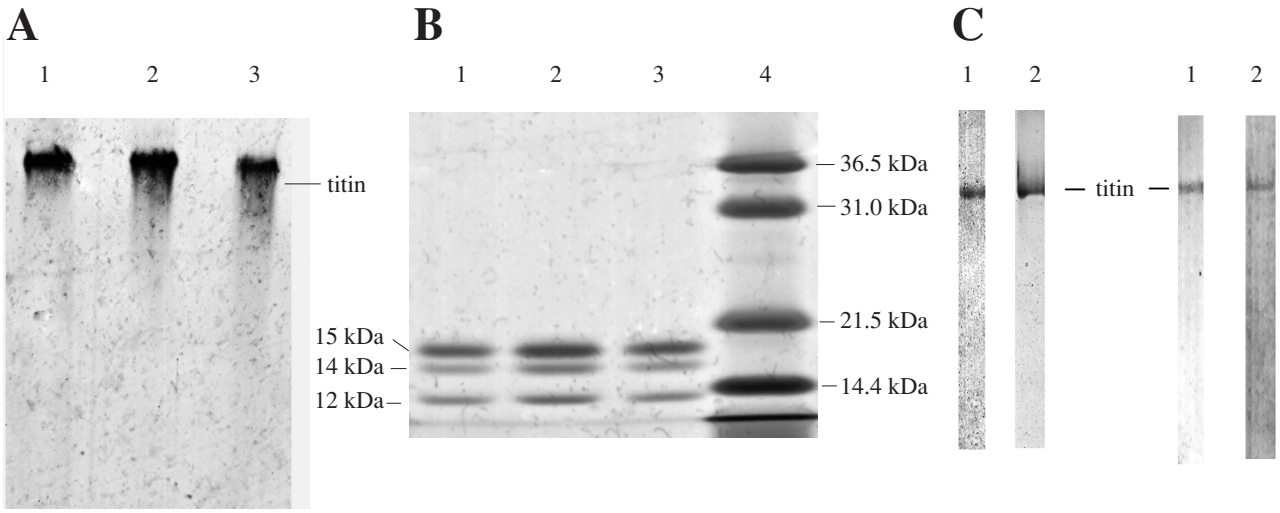

Fig. 2 Titin fractions from Sephacryl S-500 gel filtration column chromatography analyzed by gradient gel electrophoresis and SDS-PAGE. The amount of protein loaded onto each lane is 1.0 μg. (A) 2-12% (3:1) gradient gel: 1, fraction corresponding to an elution volume of 54-57 ml; 2, fraction corresponding to an elution volume of 66-69 ml; 3, fraction corresponding to an elution volume of 78-81 ml. (B) 12% SDS-PAGE: 1-3, titin fractions as described in (A); 4, Mark 12™ molecular weight standard (Invitrogen, Carlsbad, CA, U.S.A.). (C) left: Commassie blue-stained nuclear titin (Lane 1) and skeletal muscle titin (Lane 2); right: immunoblot of nuclear titin (Lane 1) and skeletal muscle titin (Lane 2) detected with a monoclonal antibody (T11) against skeletal muscle titin.

gel with trypsin and the resulting peptide mixtures were analyzed by MALDI-TOF MS.

The MS profile of the 12 kDa protein showed multiple peaks ranging from 900 to 2200 Da (Fig. 3A). Prominent peaks were selected for comparison with established databases. The protein exhibiting the highest correlation with the 12 kDa protein was found to be histone H4, corresponded to 59% sequence coverage. The peptides detected and matched to H4 histone are summarized in Table 1. To further characterize the internal sequence of the 12 kDa protein, the signals at 1325.75 Da and 1180.62 Da were selected for MS/MS analysis. The generated fragment ion spectrum identified the1325.75 Da peptide as DNIQGITKPAIR (Fig. 3B), equivalent to residues 25-36 of bovine histone H4. The 1180.62 Da peptide was identified as ISGLIYEETR (Fig. 3C), equivalent to residues 47-56 of bovine histone H4. The sequence of bovine H4 histone was used for comparison because of the incomplete sequence information of rabbit H4 histone and the fact that H4 histone is nearly identical in sequence in all eukaryotes.

The 14 kDa and 15 kDa proteins were subjected to the same analyses as above. The MS profile of the 14 kDa protein is shown in Fig. 4A. It was identified as H2A histone, which corresponded to 63% sequence coverage. The peptides detected and matched to H2A histone are summarized in Table 2. The signals at 944.53 Da and 1692.91 Da were selected for MS/MS analyses. The 944.53 kDa peptide was identified as AGLQFPVGR (Fig. 4B), equivalent to residues 24-32 of rabbit H2A histone. The 1692.91 Da peptide was identified as HLQLAIRNDEELNK (Fig. 4C), equivalent to residues 83-96 of bovine H2A histone. The MS profile of the 15 kDa protein is shown in Fig. 5A. It was identified as H3 histone, which corresponded to 57% sequence coverage. The peptides detected and matched to H3 histone are summarized in Table 3. The signals at 1032.58 Da and 1335.70 Da were selected for MS/MS analyses. The 1032.58 Da peptide was identified as YRPGTVALR (Fig. 5B), equivalent to residues 42-50 of rabbit H3 histone. The 1335.70 Da peptide was identified as EIAQDFKTDLR (Fig. 5C), equivalent to residues 74-84 of rabbit H3 histone.

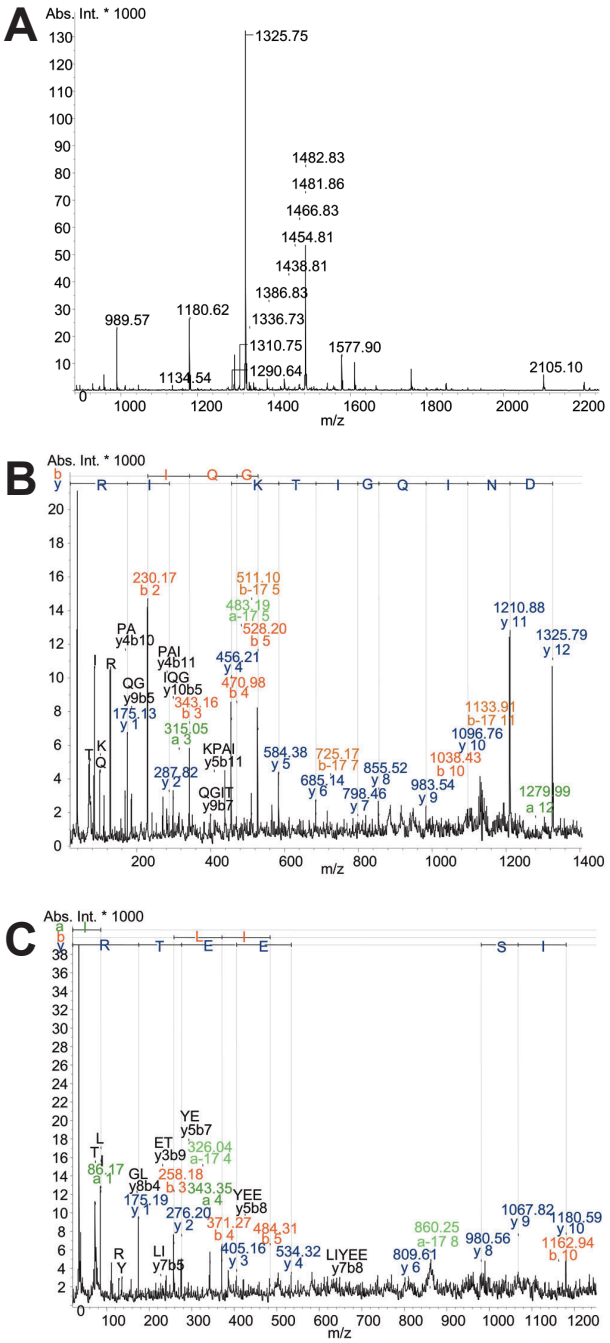

Fig. 3 Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) profile of tryptic digests of the 12 kDa protein band. (A) The 12 kDa band was digested with trypsin, the peptides were analyzed by MALDI-TOF MS, and prominent mass peaks were chosen for the database search. (B) MS/MS spectrum of the derivatized tryptic peptide with a mass of 1325.75 Da. (C) MS/MS spectrum of the derivatized tryptic peptide with a mass of 1180.62 Da.

Table 1. Monoisotopic Mass of Tryptic Peptides Derived from H4 Histone

Monoisotopic mass	Peptide (position)		Peptide sequence
Observed	Start	End	
989.5739	61	68	VFLENVIR
1134.5410	69	78	DAVTYTEHAK
1180.6173	47	56	ISGLIYEETR
1290.6413	69	79	DAVTYTEHAKR
1310.7470	81	92	TVTAMDVVYALK
1325.7502	25	36	DNIQGITKPAIR
1336.7324	46	56	RISGLIYEETR
1386.8322	57	68	GVLKVFLENVIR
1438.8126	80	92	KTVTAMDVVYALK
1454.8059	80	92	KTVTAMDVVYALK+ Oxidation (M)
1466.8270	81	93	TVTAMDVVYALKR
1481.8609	25	37	DNIQGITKPAIRR
1482.8278	81	93	TVTAMDVVYALKR+ Oxidation (M)
1577.9005	47	60	ISGLIYEETRGVLK
2105.0964	61	78	VFLENVIRDAVTYTEHAK

DISCUSSION

This study provided direct evidence for the presence of titin in the nucleus by purifying titin from the nucleus. Furthermore, we provided the first evidence demonstrating the strong interaction between titin and histones.

There were two major obstacles for the isolation and purification of nuclear titin. The first obstacle came from the exceedingly high susceptibility of nuclear titin to degradation. Effective protease inhibition was needed in all the solutions for a successful preparation. A failure to detect nuclear titin in HeLa cell extract might result from insufficient inhibition of proteases.[17] The second obstacle was the use of the appropriate gel system to analyze titin during

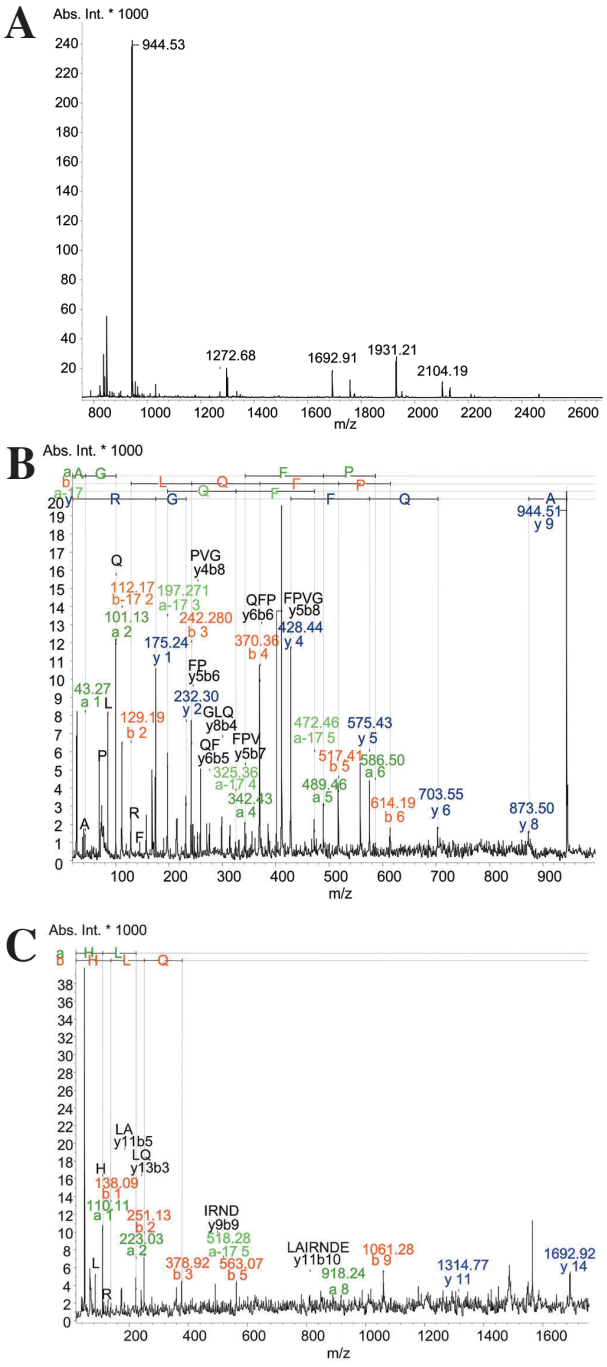

Fig. 4 MALDI-TOF MS profile of tryptic digests of the 14 kDa protein band. (A) The 14 kDa band was digested with trypsin, the peptides were analyzed by MALDI-TOF MS, and prominent mass peaks were chosen for the database search. (B) MS/MS spectrum of the derivatized tryptic peptide with a mass of 944.53 Da. (C) MS/MS spectrum of the derivatized tryptic peptide with a mass of 1692.91 Da.

Table 2. Monoisotopic Mass of Tryptic Peptides Derived from H2A Histone

Monoisotopic mass	Peptide (position)		Peptide sequence
Observed	Start	End	
944.5337	22	30	AGLQFPVGR
1272.6822	90	100	NDEELNKLLGK
1692.9108	83	96	HLQLAIRNDEELNK
1931.2061	101	119	VTIAQGGVLPNIQAVLLPK
2104.1867	83	100	HLQLAIRNDEELNKLLGK

purification. We used a 2-12% (3:1) gradient gel system in this study. This system greatly increases the large pore region that facilitates the movement of titin in the gel. However, this gel is soft and breakable which makes it extremely difficult to handle.

In muscle, titin is thought to act as a molecular scaffold for sarcomere assembly by specifying the precise position of its interacting proteins within each half sarcomere. Analogous to its role in muscle, titin may localize to chromosomes and provide a template for the correct binding and assembly of other proteins involved in chromosome condensation. Topoisomerase II and SMC were identified as the two most abundant chromosomal scaffold proteins and were proposed to determine the characteristic shape of mitotic chromosomes.[25,26] If titin is a part of the chromosomal scaffold, why wasn't it identified in the initial biochemical analysis of scaffold proteins? There are three possible explanations. The first is the easy degradation of titin as discussed in the previous paragraph. The second explanation is the size of titin. Scaffold proteins are usually identified with traditional SDS-PAGE, which does not resolve proteins of molecular weights greater than 500 kDa even when the percentage of gel is decreased to 6%. The third explanation is the easy oxidation of titin. Intermolecular disulfide bonds link titin into oligomers that are too large to enter the gel. Protein samples have to be treated with 50 mM DTT and heated at 60°C for at least 8 min to reduce the

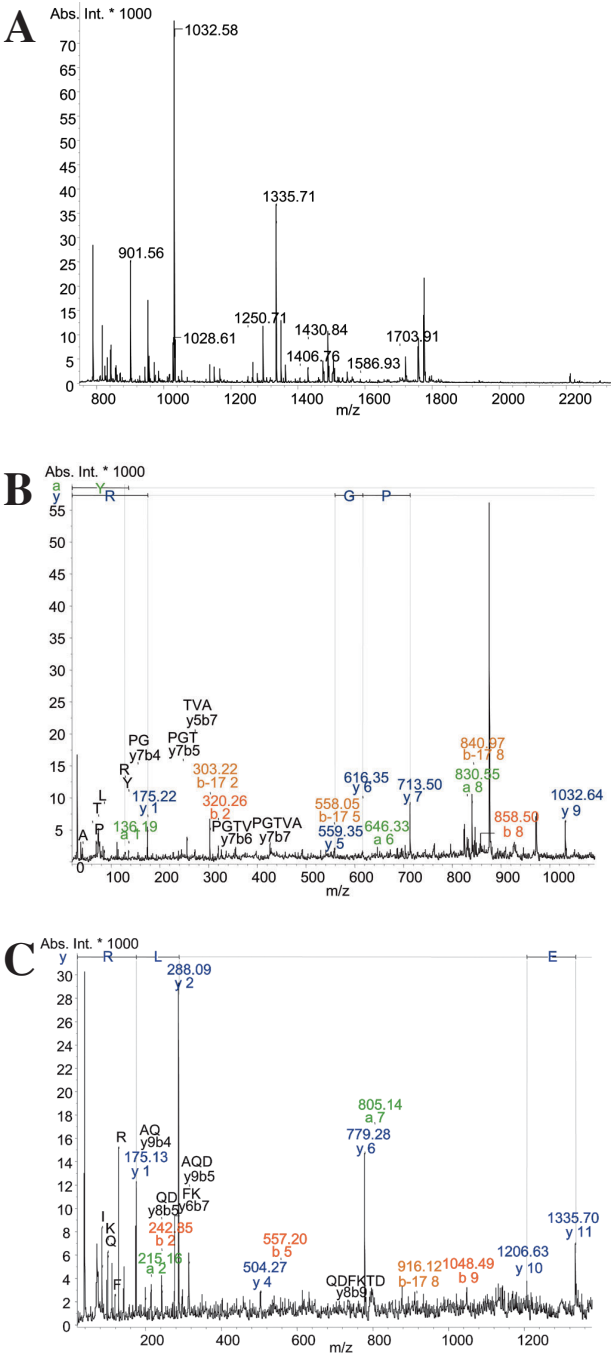

Fig. 5 MALDI-TOF MS profile of tryptic digests of the 15 kDa protein band. (A) The 15 kDa band was digested with trypsin, the peptides were analyzed by MALDI-TOF MS, and prominent mass peaks were chosen for the database search. (B) MS/MS spectrum of the derivatized tryptic peptide with a mass of 1032.58 Da. (C) MS/MS spectrum of the derivatized tryptic peptide with a mass of 1335.70 Da.

Table 3. Monoisotopic Mass of Tryptic Peptides Derived from H3 Histone

Monoisotopic mass	Peptide (position)		Peptide sequence
Observed	Start	End	
901.5618	11	19	STGGKAPRK
1028.6050	66	73	LPFQRLVR
1032.5842	42	50	YRPGTVALR
1250.7070	55	64	YQKSTELLIR
1335.7051	74	84	EIAQDFKTDLR
1406.7574	54	64	RYQKSTELLIR
1430.8400	42	53	YRPGTVALREIR
1586.9265	42	54	YRPGTVALREIRR
1703.9076	71	84	LVREIAQDFKTDLR

intermolecular disulfide bonds before loading.

The MS and MS/MS analyses clearly indicated that the inseparable partners for nuclear titin are three types of histones: H2A, H3, and H4. The DNA in chromatin is very tightly associated with histones, which package DNA into structural units called nucleosomes. Each nucleosome contains eight histone molecules, two copies each of H2A, H2B, H3 and H4. In addition, our spectroscopic study also indicated the association of nuclear titin with DNA (unpublished results). Therefore, two major components of chromatin interact with titin. These results further supported the role of nuclear titin as a chromosome scaffold. Further studies are needed to unravel the nature of interaction between titin and histones to elucidate the detailed mechanism. In addition, direct evidence is not yet available to show that nuclear titin binds to histones in vivo. The development of a specific high-titer antibody will facilitate further study in this direction. The information presented in this paper, although in the preliminary stage, provides future direction for studying the role of titin in the nucleus.

Acknowledgements

The authors are extremely grateful to Dr. Meei Jyh Jiang for valuable critical reading of the manuscript. This work was supported by Ministry of Education Grant EMRPD 180101, Taiwan, R.O.C.

REFERENCES

1. Koshland D, Strunnikov A. Mitotic chromosome condensation. Annu Rev Cell Dev Biol 1996;12:305-33.
2. Warburton PE, Earnshaw WC. Untangling the role of DNA topoisomerase II in mitotic chromosome structure and function. Bioessays 1997;19:97-9.
3. Houchmandzadeh B, Dimitrov S. Elasticity measurements show the existence of thin rigid cores inside mitotic chromosomes. J Cell Biol 1999;145:215-23.
4. Strunnikov AV, Jessberger R. Structural maintenance of chromosomes (SMC) proteins: conserved molecular properties for multiple biological functions. Eur J Biochem 1999;263:6-13.
5. Gregorio CC, Granzier H, Sorimachi H, Labeit S. Muscle assembly: a titanic achievement? Curr Opin Cell Biol 1999;11:18-25.
6. Tskhovrebova L, Trinick J. Titin: properties and family relationships. Nat Rev Mol Cell Biol 2003;4:679-89.
7. Tskhovrebova L, Trinick J. Properties of titin immunoglobulin and fibronectin-3 domains. J Biol Chem 2004;279:46351-4.
8. Labeit S, Kolmerer B. Titins: giant proteins in charge of muscle ultrastructure and elasticity. Science 1995;270:293-6.
9. Puchner EM, Alexandrovich A, Kho AL, Hensen U, Schäfer LV, Brandmeier B, Gräter F, Grubmüller H, Gaub HE, Gautel M. Mechanoenzymatics of titin kinase. Proc Natl Acad Sci USA 2008;105:13385-90.
10. Lange S, Ehler E, Gautel M. From A to Z and back? Multicompartment proteins in the sarcomere. Trends Cell Biol 2006;16:11-8.
11. Linke WA. Sense and stretchability: the role of titin and titin-associated proteins in myocardial stress-sensing and mechanical dysfunction. Cardiovasc Res 2008;77:637-48.
12. Machado C, Sunkel CE, Andrew DJ. Human autoantibodies reveal titin as a chromosomal protein. J Cell Biol 1998;141:321-33.
13. Fabian L, Xia X, Venkitaramani DV, Johansen KM, Johansen J, Andrew DJ, Forer A. Titin in insect spermatocyte spindle fibers associates with microtubules, actin, myosin and the matrix proteins skeletor, megator and chromator. J Cell Sci 2007;120(Pt 13):2190-204.
14. Machado C, Andrew DJ. D-Titin: a giant protein with dual roles in chromosomes and muscles. J Cell Biol 2000;151:639-52.
15. Zastrow MS, Flaherty DB, Benian GM, Wilson KL. Nuclear titin interacts with A- and B-type lamins in vitro

and in vivo. J Cell Sci 2006;119(Pt 2):239-49.
16. Qi J, Chi L, Labeit S, Banes AJ. Nuclear localization of the titin Z1Z2Zr domain and role in regulating cell proliferation. Am J Physiol Cell Physiol 2008;295:C975-85.
17. Takata H, Uchiyama S, Nakamura N, Nakashima S, Kobayashi S, Sone T, Kimura S, Lahmers S, Granzier H, Labeit S, Matsunaga S, Fukui K. A comparative proteome analysis of human metaphase chromosomes isolated from two different cell lines reveals a set of conserved chromosome-associated proteins. Genes Cells 2007;12:269-84.
18. Harris ELV, Angal S. Protein purification methods, a practical approach. 1st ed. NY, USA: Oxford University Press, 1989:97-101.
19. Busch H, Daskal Y. Methods for isolation of nuclei and nucleoli. Methods Cell Biol 1977;16:1-43.
20. Fritz JD, Swartz DR, Greaser ML. Factors affecting polyacrylamide gel electrophoresis and electroblotting of high-molecular-weight myofibrillar proteins. Anal Biochem 1989;180:205-10.
21. Warren CM, Krzesinski PR, Greaser ML. Vertical agarose gel electrophoresis and electroblotting of high-molecular-weight proteins. Electrophoresis 2003;24:1695-702.
22. Knight P, Offer G. p-NN'-phenylenebismaleimide, a specific cross-linking agent for F-actin. Biochem J 1978;175:1023-32.
23. Hu DH, Kimura S, Maruyama K. Cross-linking of native myosin forms oligomers of myosin heavy chain dimers. J Biochem 1988;104:509-11.
24. Shevchenko A, Tomas H, Havlis J, Olsen JV, Mann M. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat Protoc 2006;1:2856-60.
25. Kimura K, Hirano T. ATP-dependent positive supercoiling of DNA by 13S condensin: a biochemical implication for chromosome condensation. Cell 1997;90:625-34.
26. Sutani T, Yanagida M. DNA renaturation activity of the SMC complex implicated in chromosome condensation. Nature 1997;388:798-801.

彈性連結素在細胞核內與組蛋白作用

金 蘭　周純如

背 景： 彈性連結素是一條巨大的蛋白質長鏈，爲肌肉收縮的彈性元件，也是使肌肉中各種蛋白質能正確排列組合的模架。他在細胞核中是否存在，至今尚未有定論。

方 法： 從細胞核萃取蛋白質後以硫酸胺沉澱分離，再用管柱色層分析法純化彈性連結素。最後用質譜 (MS 和 MS/MS) 鑑定那些與彈性連結素無法分離的小蛋白質。

結 果： 這個純化方法可從細胞核中純化出純度不錯的彈性連結素，只是有三個小蛋白質分子經由各種色層分析管柱都無法與彈性連結素分開，顯示它們和彈性連結素之間有極強的作用。質譜鑑定這些小蛋白質是三種組蛋白：H2A、H3 和 H4。

結 論： 因爲可以從細胞核中純化出彈性連結素，所以可以肯定它存在於細胞核。而從它與組蛋白的密切結合，可以推測它在染色體的模架角色。

(長庚醫誌 2010;33:201-10)

關鍵詞：彈性連結素，細胞核，蛋白質純化，組蛋白，蛋白質相互作用，蛋白體學

長庚大學 醫學院 生化及分子生物學科
受文日期：民國98年4月28日；接受刊載：民國98年6月4日
通訊作者：金蘭副教授，長庚大學 生化及分子生物學科。桃園縣333龜山鄉文化一路259號。
Tel.: (03)2118800轉5159; Fax: (03)2118452; E-mail: lanking@mail.cgu.edu.tw