Tumor Res. **30**, 21—36 (1995)

Isolation of cDNA Encoding 7H6-Reactive Polypeptide Defines a New Class of Protein with α-Helical Coiled-Coil Structure and DA-Box Similar to Yeast Chromosomal Segregation Proteins

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ABSTRACT

7H6 monoclonal antibody was recently developed in our laboratory by immunizing mice with a bile canaliculus-rich fraction of the rat liver. The antibody reacted with a novel 155 Kd polypeptide designated 7H6 antigen that specifically localizes at tight junctions of various epithelia. Correlations of the paracellular barrier function of the tight junction with expression of the 7H6 antigen at the cell border have suggested important roles of this polypeptide for the maintenance of tight junctional functions. As the first step for the analysis of the antigen at the molecular level, we isolated a series of cDNA clones encoding 7H6-reactive polypeptides. Five clones were isolated by immunoscreening. Among them a clone designated RL5.3 which carries the largest 5.3Kb insert was characterized in this study. Both plaque screening and immunoblotting of the fusion protein produced by the RL5.3 clone with lysogen confirmed that the protein specifically reacts with the 7H6 monoclonal antibody. Using DNA fragments

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Abbreviations used are :

cDNA, complementary DNA
mAb, monoclonal antibody
NFDM, non-fat dry milk
pfu, plaque forming unit
PAGE, polyacrylamide gel electrophoresis
PCR, polymerase chain reaction
PBS, phosphate buffered saline
TER, transepithelial electrical resistance
LB, Luria-Betrtani medium
SDS, sodium dodecyl sulfate.

of the RL5.3 clone, 21 clones were further identified. Studies with restriction enzymes and probe hybridization revealed that all the cDNA clones were derived from a single class of transcripts. A partial sequence identified one open reading frame with an α-helical coiled-coil structure and highly conserved aspartate (D)- alanine (A) residues with a helix-loop-helix structure corresponding to DA- box. Since this domain has been specifically found in yeast chromosomal segregation proteins (*SMC1, CUT3 and CUT14*), the polypeptide encoded by the RL5.3 clone provides the first rodent counterpart of these protein family. Yeast is known to be lethal when SMC and CUT proteins are deleted, suggesting essential roles of these proteins for cell cycle progression as a regulator for chromosomal segregation. Identification of a mammalian counterpart of this protein family may give us some clues for a better understanding of fundamental regulatory mechanisms in the function of tigh junctions.

key words: α-helical coiled-coil structure, DA-box, monoclonal antibody, tight junction, Walker's B site.

INTRODUCTION

The tight junction functions as a paracellular barrier for solutes, small molecules and immune cells, and also serves as a fence which separates the apical and basolateral membrane compartments in epithelial and endothelial cells(1, 2). Many works have been directed to elucidate the regulatory mechanisms of the tight junction, such as the assembly and the barrier function, at the molecular level(3, 4, 5, 6, 7, 8).

By immunizing mice with a bile canaliculus-rich fraction isolated from rat liver, we have generated the 7H6 monoclonal antibody, which reacts with a novel protein component of the tight junction. The protein masses approximately 155 Kd on SDS-PAGE(9) and has been designated the 7H6 antigen. The 7H6 antigen was shown to localize preferentially at tight junctions of MDCK cells when the tight junction was resistant to the passage of ions and macromolecules, whereas it disappeared from the cell border when the paracellular barrier functions were perturbed by experimental ATP depletion(10). Furthermore, induction of 7H6 antigen at tight junctions of rat lung endothelial (RLE) cells by treatment with dibutyryc cyclic AMP or all-trans-retinoic acid enhanced paracellular barrier function of these cells, and significantly reduced the rate of tumor cell transmigration across the endothelial cell monolayers(11). The 7H6 antigen is expressed at the tight junction of epithelial cells in a wide variety of animals, from avians to humans, in a discrete manner(12). This evolutionally conserved presence of 7H6 antigen among Metazoa suggests unique and essential roles of

this peptide in the regulation and maintenance of tight junctions. The characteristic features of the 7H6 antigen in addition to its specific distribution at the tight junction(9, 10) have prompted us to study the molecular structure of this polypeptide.

In the present study, we screened λgt11 expression library prepared from rat liver and identified a series of cDNAs which produced polypeptides reactive to the 7H6 monoclonal antibody. Studies of these λ clones revealed that all were derived from a single class of transcripts in the rat liver. Amino acid sequences deduced from partial nucleotide sequences revealed that the polypeptide contains an α-helical coiled-coil structure and a putative ATPase domain. A high structural homology was found between the polypeptide and the protein product of yeast chromosomal segregation genes. These results suggested that the 7H6 reactive polypeptide may be the first counterpart of chromosomal segregation protein family in rodent. The significance of 7H6 polypeptide in mammalian cells is briefly discussed.

MATERIALS AND METHODS

Reagents

All reagents were purchased from Wako Chemicals (Osaka, Japan) unless otherwise indicated.

Isolation and characterization of cDNA encoding 7H6 reactive polypeptides

λgt11 expression library of rat liver (code. RL3000b) was purchased from CLONTECH and screened with culture supernatant of 7H6 producing hybridoma cells(8). The appropriate titer was examined before library screening with immunoblotting using plasma membrane fraction of MDCK cells as the source of 7H6 antigen(13, 14). Screening was performed with a lytic strain of E. coli, Y1090 at $3\text{-}5\times10^4$ pfu by plating in 9 cm bacterial dishes and allowed to grow for 3.5 hrs at 42℃. Nitrocellulose filters pre-soaked in 10 mM IPTG were overlayed on the bacterial plates. These plates were incubated for another 4 hrs at 37℃. Nitrocellulose filters were pre-washed in TNT buffer (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Triton-X100) several times and non-specific binding of antibodies was blocked by 10% NFDM in PBS. Filters were then reacted with 7H6 monoclonal antibody, and processed using goat anti-mouse immunoglobulin conjugated with alkaline phosphatase (×1000 dilution)(DAKO A/S, Denmark). Antigen-alkaline phosphatase complex was visualized using 5-bromo-4-chloro-3-indol phosphate (BCIP) in combination with nitro blue tetrazolium (NBT) supplied as a kit (alkaline phosphatase substrate kit) by Vector Laboratories Inc. (Burlingame, CA).

Purification of λ DNA

Purified λ clones were amplified to obtain high titer stocks. These clones were used for isolation of λ DNA according to previous reports(15, 16, 17). Briefly, 10^9 pfu was infected with exponentially growing Y1090 cells in 50 m*l* LB medium containing 10 mM $MgSO_4$ and cultured until lysis was occurred. Liquid lysates of each λ clones were precipitated with 7.5% polyethylene glycol and 0.75 M NaCl at 4℃. Precipitated phages were incubated in 0.2% SDS, 5 mM Tris-HCl pH 7.5, 5 mM NaCl, 1.25 mM EDTA for 20 min at 70℃. After centrifugation to remove precipitates, supernatants were subjected for DNA purification using QIAGEN resin (QIAGEN Lamda, Hilden, Germany). Purified DNA was suspended in TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA) until use.

Preparation of λgt11 lysogens and immunoblotting

Using a clone containing 5.3 Kb insert, which was isolated and purified for the specific binding of 7H6 monoclonal antibody as described above, fusion proteins encoded by λgt11 lysogen were prepared and characterized by immunoblotting. Lysogens were prepared as described(13, 14). 10^{10} pfu/ml of λ clone (RL5.3) on Y1090 hsdR was infected to Y1089 and aliquots of infectants were cultured on LB plates containing ampiciline (50 μg/m*l*). Each colony was then transferred and allowed to grow at 30℃ or 42℃ for 12-16 hrs. Colonies grown at 30℃ but not at 42℃ were picked up as lysogenic recombinants. These lysogens were used for transduction of fusion protein in the presence of isopropylthio-β-D-galactoside (IPTG) at a concentration of 10 mM. Lysogenic cultures were spun down and lyzed with SDS-sample buffer for immunoblotting. Immunoblotting was performed as described except that electro-transfer was done using semi-dry electro blotter (ATTO, Tokyo, Japan) at 150 mA for 20 min onto Trans-blot (Bio-Rad, Hercules, CA) nitrocellulose membrane(9).

Preparation of hybridizing filters for phage clones.

For plaque hybridization, positively charged colony/plaque screen (NEN Research Products, Boston, MA) nylon membranes, were transferred onto bacteriophages. DNAs were denatured with 0.2N NaOH, neutralized with 1 M Tris-HCl pH 7.5 and fixed by air-drying according to the manufacturer's recommendation.

Preparation of DNA blotts

λ DNA was prepared as described above. One μg of DNA was cleaved with an appropriate endonuclease and electrophoresed in 0.9% agarose gel containing

40 mM Tris-acetate pH 7.2, 1 mM EDTA and 0.4 μg/ml of ethidium bromide. DNA gel was soaked in denaturation buffer (1.5 M NaCl and 0.5 M NaOH) and neutralized in 1.5 M NaCl, 0.5 M Tris-HCl pH 7.2 and 1 mM EDTA.

Hybridization of plaque and DNA filters

DNA separated on an appropriate gel was transferred to Gene Screen Plus nylon membrane (NEN Research Products, Boston, MA) by capillary action using 10× SSC(16). DNA fragments were labeled with [α-^{32}P] dCTP by primer extension (Amersham LIFE SCIENCE, UK) and used at 1-10×10^{5}cpm/ml in a hybridization buffer. DNA and plaque filters were immersed in pre-hybridization buffer containing 50% formamide, 2× SSC, 1% SDS, 10% dextran-sulfate and 100 μg/ml seared salmon sperm (ssDNA). After hybridization, filters were washed at a high stringent condition (2× SSC, 1% SDS at 65℃) and exposed to X-ray film (X-AR, Kodak, Rochester, NY) or imaging plates of the BAS2000 system (FUJI, Tokyo, Japan).

Nucleotide sequencing, sequencing analysis and amino acid analysis

Nucleotide sequences of double stranded plasmid carrying various fragments of RL5.3 clone were determined by Sanger's dideoxy chain termination using taq DNA polymerase and fluorescent primers supplied from Perkin-Elmer Co. (Foster City, CA). Sequences were analyzed by 373S automated sequencer (Perkin-Elmer Co.). Nucleotide sequences and deduced amino acid sequences were analyzed with a GENETYX DNA analysis software package (CDC software Co, Tokyo, Japan) and the COILCOIL program(18), BLASTN and BLASTP on the Sapporo Medical University Computer Network (Sapporo, Japan); these are now available via the internet.

Reverse transcription PCR (RT-PCR)

Total cellular RNA was extracted by acid guanidium thiocyanate-phenol-chloroform (AGTPC) supplied as Isogen (Nippon Gene, Toyama, Japan). Tissues or culture cells were suspended in AGTPC solution at a weight ration of 1 : 100 and homogenized with polytron. Total RNA was then isolated by spinning at 10,000 g in the presence of chloroform. RNA precipitates were dissolved in RNase free water. To isolate a part of ZO-1 cDNA, RT-PCR was performed on rat liver RNA as described(4) using RNAPCR kit (Perkin-Elmer Co.). Primers used were, forward; 5'-CATAGAATAGACTCCCCTGG-3' (2194), reverse; 5'-GCTTGAGGACTCGTATCTGT-3' (2196). Amplified cDNA was purified by ethanol precipitation and was used as a probe for hybridization. Other molecular techniques including restriction enzyme digestion were performed

as described(16).

RESULTS

Isolation of λgt11 clones expressing 7H6 reactive polypeptides

Rat liver cDNA library was screened with 7H6 monoclonal antibody. Of 1.2×10^6 λgt11 clones, 5 were found to be positive. These clones were further purified by 3 to 4 rounds of plaque purification. λ DNA was purified from a high titer of liquid lysate and subjected to a restriction enzyme study. Three (clone names RL5.3/1, RL5.3/4 and RL5.3/5) out of the 5 were shown to carry about 5.3 Kb EcoRI fragment. Other clones contained a 2.2 Kb (RL2.2) and a 1.7 Kb (RL1.7) fragment. Comparison of restriction enzyme patterns (Fig. 2 and data not shown) revealed that these clones shared identical fragments, indicating that all the clones obtained were derived from a single class of cDNA. Therefore, we concentrated on characterizing the RL5.3/1 clone (referred as RL5.3, hereafter)

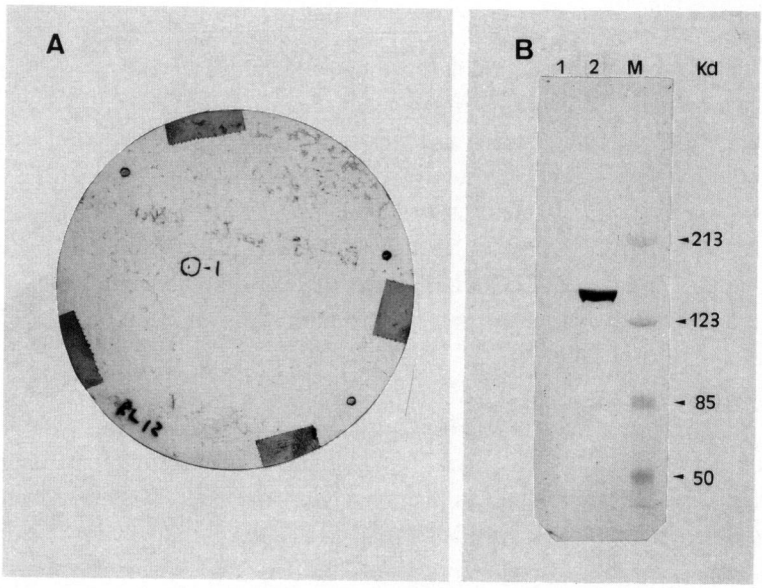

Fig. 1 Reactivity of 7H6 monoclonal antibody for λ clones.
A. A representative positive signal with 7H6 monoclonal antibody for λgt11 plaques constructed with rat liver cDNA.
B. Immunoblotting of fusion protein expressed by lysogen without insert(1) or with RL5.3 insert(2). Specific staining with 130 Kd polypeptide was depicted in lane 2. M: molecular weight marker (from the top to bottom, myosin; 213 Kd, β-galactosidase; 123 Kd, Bovine serum albumin; 85 Kd, Ovalbumine; 50 Kd).

which contains the largest 5.3 Kb insert. To ensure the authenticity of immunoscreening, plaque-purified RL5.3 was transduced to synthesize fusion protein with a lysogenic E.coli strain, Y1089 (Fig. 1A and 1B). Total cell extract was resolved in SDS-PAGE sample buffer and studied by immunoblotting. A 130 Kd polypeptide was specifically detected with 7H6 mAb (Fig. 1B lane 2). No apparent band was observed with Y1089 lysate in control λ clone (no insert)(Fig. 1B lane 1). Specific binding of 7H6 mAb was confirmed using bacterial fusion protein produced by a pMAL expression vector with JM109 E. coli strain (data not shown). This specific binding of 7H6 mAb with the recombinant 130 Kd protein and monospecificity of the monoclonal antibody strongly suggested that isolated cDNA encodes a 7H6-reactive polypeptide.

Isolation and characterization of λ clones by immuno- or DNA probe revealed

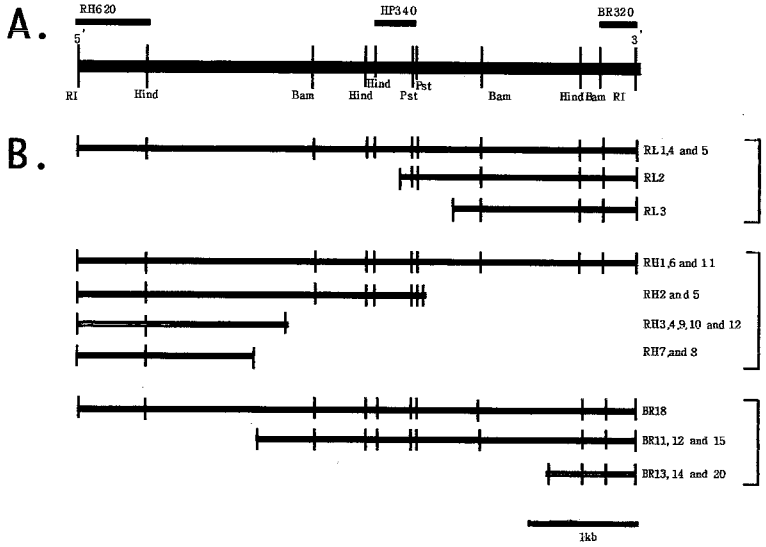

Fig. 2 Restriction map of RL5.3 cDNA insert and its relative site for isolated clones with either immunoscreening or nucleic acid probing.

A. Cleavage sites of each restriction enzyme (RI; EcoRI, Hind; HindIII, Bam; BamHI, Pst; PstI) were schematically shown. Relative positions of DNA fragments used for hybridization were also inserted as RH620, HP340 and BR320 with detached fat line.

B. Clones isolated with 7H6 monoclonal antibody by immunoscreening (RL1-5), or nucleic acid probing with RH620 (RH1-12) or with BR340 (11-15, 18 and 20) were aligned in order to identify their identical restriction fragments. The results indicated all the clones were derived from a single class of transcripts.

identical derivation from a single class of cDNA from two independent libraries.

Based on the restriction fragment pattern (as depicted in Fig. 2A and data not shown), we isolated three cDNA fragments, RH620, HP340 and BR320 from the most 5', the middle and the most 3' portion of the RL5.3 cDNA, respectively. Using RH620 and BR320, two independent rat liver libraries, λgt11 (RL3000b) and a λgt10 library (RL1023a) were screened. With the RH620 probe, 12 clones were isolated, whereas the BR320 identified 7 clones. Purified λ DNAs were analyzed by restriction enzyme digestion and Southern blot hybridization. To evaluate the quality of each library, the frequency of positive clones with different probes was studied and the result was summarized in Table 1. When the rat liver λgt10 or the λgt11 library was screened with human β-actin probe, 0.14 to 0.22% of clones were hybridized. The percentage of plaques to β-actin is considered to be a useful marker for normal sequence distribution. The frequency of β-actin positive plaques was within a range of reasonable frequency (0.10-0.90%). ZO-1 probes were also used for the plaque hybridization which showed that 0.052-0.094% plaques were hybridized. Using identical plaque filters, RH620 and BR320 probes were hybridized. 0.01-0.008% of plaques were found to be positive. This study exhibited relative frequency of β-actin, ZO-1 and the RL5.3 cDNA clone in these libraries, though the abundancy of mRNA may not directly correspond to that of positive plaques in the library. The relative frequency of RL5.3 clone was low, which may indicate a rare transcript in the tissue of rat liver.

λ clones isolated by immunoscreening and hybridization screening were purified for DNA. Restriction enzyme digestion and Southern blot analysis were performed. The localization of each restriction enzyme and fragment lengths are schematically shown in Fig. 2B. As described above, immunoscreening revealed

Table 1 *Frequency of positive signals in two liver cDNA libraries used in this study probed with β-actin, ZO-1 and RL5.3 cDNA fragments (RH620 and BR340).*

	RL3000b[a]	RL1023[b]
β-actin	0.14[c]	0.22
ZO-1	0.052	0.094
RH620(RL5.3)	0.01	0.009
BR320(RL5.3)	0.008	0.009

a: Rat liver cDNA library construction with λgt11 vector.

b: Rat liver cDNA library constructed with λgt10 vector.

c: Each value was shown as % of positive signal with each probe per 10^5 plaques in each hybridization experiment.

three types of λ clones containing different size of cDNA insert. RH620 hybridized four different types of cDNA inserts including 5.3 Kb (RH1, 6 and 11), 3.3 Kb (RH2 and 5), 2.0 Kb (RH3, 4, 9, 10 and 12) and 1.4 Kb (RH7 and 8). BR320 identified three types of cDNA clones, 5.3 Kb (BR18), 3.6 Kb (BR11, 12 and 15) and 0.8 Kb (BR13, 14 and 20). HindIII+EcoRI or BamHI+EcoRI enzyme cleavage was used to prepare Southern blotting. These filters were hybridized with RH620, HP340 and BR320 probes. This study exhibited common cDNA fragments among these clones. Summary of the restriction enzyme mapping and hybridization study in Figure 2A showed that all of these clones were derived from a single class of cDNA in two independent cDNA libraries. The observation of identical cDNA clones in the two independent libraries poses the possibility that these clones were representatives of naturally occurring transcription in the rat liver.

Partial amino acid sequences deduced from nucleotide sequences of RL5.3 cDNA

Various fragments of RL5.3 digested with restriction enzymes were subcloned into pBluescript II KS(−). These plasmids were purified by CsCl gradient with ultracentrifugation and used for cycle sequencing reaction. Each plasmid was sequenced from both ends of subcloning sites at least twice. Junctional sites of restriction enzymes were sequenced using synthetic primers corresponding to nucleotide sequences of the flanking region.

Nucleotide sequence revealed one open reading frame. Amino acid sequences deduced from the nucleotide sequences predicted 584 amino acids with a molecular mass of 63.3 Kd. Study using a bacterial expression vector containing RL5.3 insert demonstrated this cDNA encoded at least a 130 Kd polypeptide as shown (in Fig. 1B and data not shown). This apparently suggested that the fragment currently sequenced encoding a part of 7H6 reactive polypeptides.

The PROSITE data library (release 11.1) was searched for potential post-translational modification sites in this polypeptide. Six putative protein kinase C phosphorylation sites and ten casein kinase II phosphorylation sites were found (Fig. 3). The 7H6 protein was phosphorylated in MDCK cells(10). Possible phosphorylation sites in this peptide fragment indicated these regions were a good candidate for determining in vivo phosphorylated residues. Potential N-glycosylation sites were also important to modify this polypeptide, since 7H6 reacted with different sizes of polypeptides depending on the type of cells studied.

Comparison of amino acid sequences with known proteins revealed the 7H6 polypeptide as the first rodent member of a gene family carrying putative ATPase together with α-helical coiled-coil structure

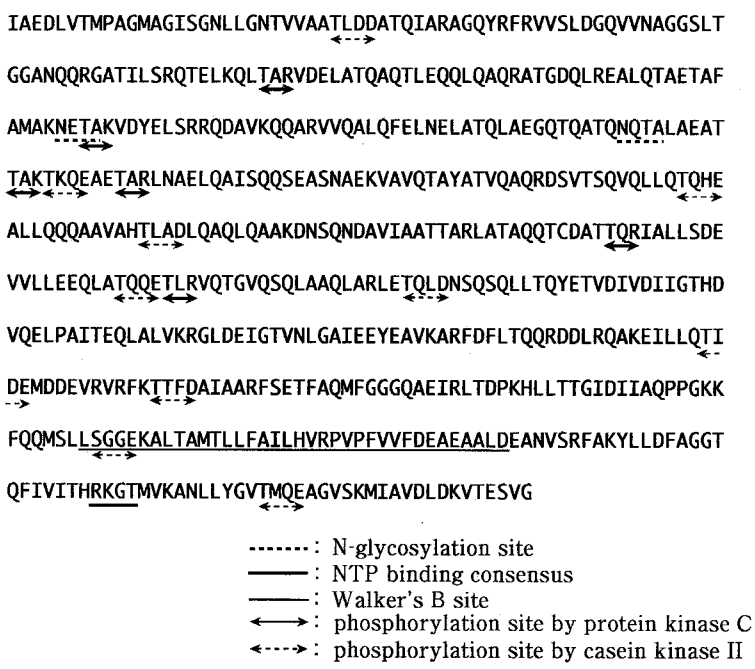

Fig. 3 Partial amino acid sequence deduced from RL5.3 nucleic acid sequencing. Putative functional motifs are also indicated.

Potential protein similarities between the 7H6 peptides and known proteins were analyzed by searching the EMBL (release 42) and SWISS-PROT (release 31) data libraries using GENETYX, BLASTP and BLASTN. No overlapping homology of the 7H6 polypeptide to any protein in the data base was found, indicating that it is a novel protein. Several motifs of the 7H6 peptide were, however, identified in known proteins of prokaryotic and eukaryotic sources (Fig. 3). A conserved sequence for putative ATP-binding (Walker's B site) was found in amino acid 548-551. A potent α-helical coiled-coil structure was predicted by the COILCOIL program in amino acid 1-584 with significant similarity to a number of proteins containing coiled-coil structure (Fig. 4). The predicted α-helical coiled-coil structure and ATP-binding site showed a strong similarity to yeast chromosomal segregation proteins. SMC1 protein, a founder and the best characterized member of this protein family, has two α-helical coiled-coil domains with two potential ATP-binding sites (Walker's A and B sites), both of which were observed in 7H6 polypeptide. Most strikingly, highly conserved aspartate (D) and alanine (A) residues were found in discrete parts of both 7H6 and SMC1 protein (see Fig. s 4 and 5). The potential helix-loop-helix structure, referred to

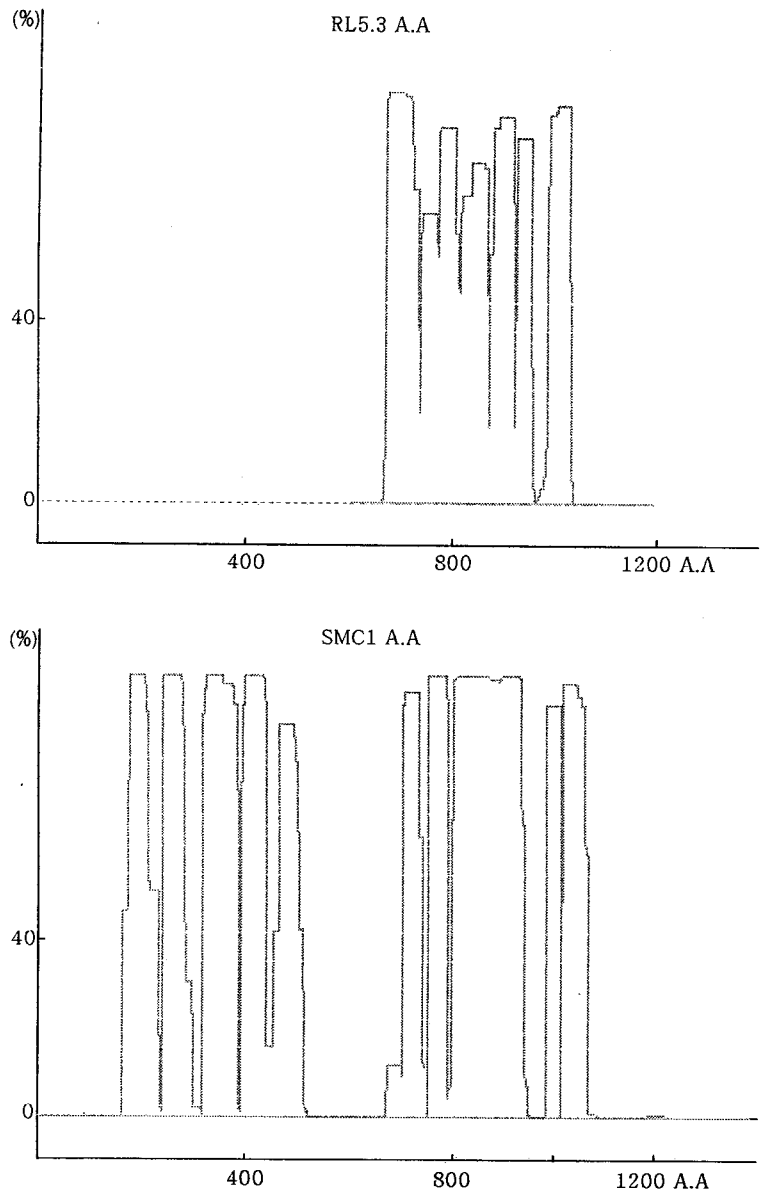

Fig. 4 An output of analysis with the COILCOIL program on partial amino acid sequence shown in Fig. 3. and its direct comparison to yeast chromosomal protein SMC1.
Amino acid sequences were searched for their potential coiled-coil structure with the COILCOIL program and revealed the α-helical coiled-coil tertiary structure in the 7H6 polypeptide with strong similarity to SMC1 protein. Numbers indicate amino acid numbers of SMC1 and do not correspond to that of RL5.3. The potent coiled-coil structure of RL5.3 was aligned for the convenience of comparison.

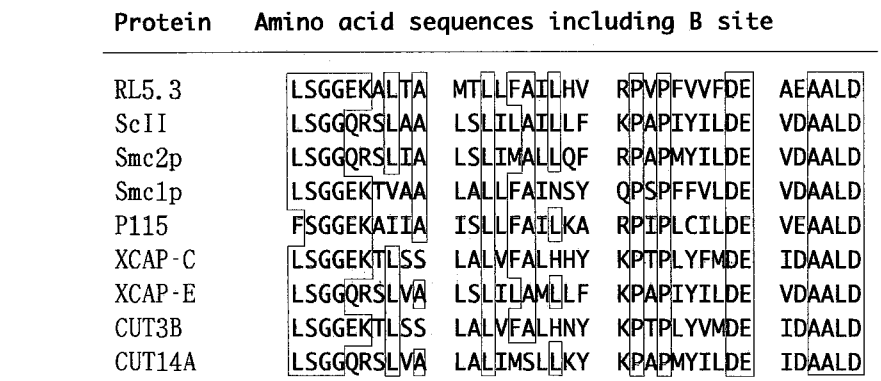

Fig. 5 Comparison of amino acid sequences of potent member of DA- box proteins.
Identical amino acids were highlighted with boxes. Strong evolutional conservation was observed among this region in all the members of this family. Origin of sequences were RL5.3; this study, ScII(20), smc1p(19) and 2p(20), P115(22), XCAP-C and E(21), and CUT3B and 14A(23).

as a DA-box, was located in a. a. 487-522 of 7H6 polypeptide and 1129-1164 of SMC1. This structure was present in all yeast chromosomal proteins and other related gene products in both prokaryotes and eukaryotes. Although the function of this portion is now unknown, its evolutionary conservation in these functionally related proteins suggested that the DA-box is indeed a hallmark of chromosomal segregation proteins in yeast, chicken, xenopus and perhaps in bacteria. Identification of a DA-box in 7H6 polypeptide demonstrated the first example of rodent counterpart of this protein family (Fig. 5).

DISCUSSION

In the present study, we attempted to isolate cDNAs which encode polypeptides reactive with the 7H6 monoclonal antibody and characterize the polypeptides. 7H6 monoclonal antibody is a new antibody recently established in our laboratory by immunizing mice with a bile canaliculus-rich fraction of the rat liver. The antibody defined a novel 155 Kd tight junction-associated protein designated 7H6 antigen which is different from previously reported tight junction proteins such as ZO-1, ZO-2 and cingulin. The antigen is found at the tight junction of various epithelial cells in a wide variety of animals, from avians to humans(9).

We isolated a series of cDNAs from two independent libraries. Since overlapping clones isolated from either by immunoscreening or with the nucleic acid probe were identical and representative from the same cDNA fragments, the

cDNA clone was considered to be a bona fide clone for the 7H6 monoclonal antibody. Then we obtained nucleotide sequences. We deduced amino acid sequences and found that the cDNA encodes a protein structurally homologous with a set of proteins designated the SMC family(19, 20, 21, 22, 23). These proteins are characterized by the presence of α-helical coiled-coil structures and putative ATPase domains corresponding to Walker's A and B sites(19, 20, 21, 22, 23). Motif analysis of 7H6 reactive polypeptide further demonstrated that this protein possesses several putative posttranslational modification sites, i. e. N-glycosylation sites, phosphorylation sites by protein kinase C and casein kinase II. The biological significance of these predicted motifs was not elucidated in the present study, but they seem quite likely to play regulatory roles in posttranslational modification for the function of 7H6 polypeptide are highly expected.

Although the functions of the members of the SMC family of proteins are still elusive, the evidence that the deletion of SMC1 function in the *ts* mutant causes lethal phenotypes during cell division in budding yeast strongly indicates that SMC1 proteins are essential for nuclear division in yeast(19). ScII protein, another SMC family member abundant in the chromosomal scaffold fraction of the chicken lymphoblastoid cell line, was shown to colocalize with topoisomerase II(20). A possible interaction of these proteins appears essential for chicken chromosome condensation, suggesting that at least some members of the SMC family interact with other family members to fulfill their functions(20). As was predicted in the study of ScII, the member of this family probably dimerize to express ATPase activity(20), since Walker's A and B sites were separated by two coiled-coil domains(24). Xenopus chromosome condensation proteins, XCAP-C and XCAP-E, are indeed shown to exist in a heterodimer form in vivo as well as in vitro systems(21). Since α-helical coiled-coil regions of each domain are predicted to serve as a homotropic aggregation signal(21), it is highly expected that heterodimer formation is a common feature of this family. The highly conserved structure in the SMC family which is also found in the 7H6 polypeptide suggests that fundamental function of these proteins might be well conserved in the 7H6 reactive polypeptide.

The α-helical coiled-coil structure is also found in other gene products besides the SMC family which are important for the arrangement of intermediate filaments. Plectin and restin commonly contain a single α-helical coiled-coil structure and play a pivotal role for the organization of intermediate filaments (25, 26). Plectin is one of the best characterized cross-linking proteins for intermediate filaments, spectrin and microtubules-associated proteins MAP 1 and 2 (25). This cross-linking seems to occur in a domain specific manner, since plectin contains three possible domains for interacting with other proteins, N-

and C-terminal globular regions and R2 coiled-coil region(25). A biochemical study indicated that plectin uses coiled-coil domain for the specific interaction with intermediate filaments(25). Restin has been identified in the Hodgkin cells and suggested to be responsible for the progression of Hodgkin's disease. This protein is shown to bind preferentially to the Reed-Sternberg cell-specific intermediate filaments through the α-helical coiled-coil domain(26). Its ATPase activity is a critical element in its role. Unlike myosin heavy chain, which possesses Walker's A and B regions close to each other(27), the 7H6 polypeptide has an α-helical coiled-coil domain adjacent to Walker's B region. This feature has strongly suggested that Walker's A site is located upstream to the coiled-coil region of 7H6 polypeptide, similar to other members of SMC family. Its potent ATPase activity may play an important role in the function of SMC family of proteins.

It is of interest that the 7H6 polypeptide lacks a nuclear localization signal sequence, whereas that sequence is well conserved among the yeast chromosomal segregation proteins. This may explain why the 7H6 polypeptide localizes mostly not in the nucleus but at the tight junction and suggests some specific adaptation during the evolution of the SMC family. Irrespective of its possible mechanism of 7H6 polypeptide in cellular events, identification of a cDNA clone encoding a 7H6-reactive polypeptide gave us an opportunity to dissect out the molecular aspect of this particular polypeptide in the tight junction as well as general cellular functions.

ACKNOWLEDGMENTS

This study was performed through Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

This work was supported in part by Grants in Aid from the Ministry of Welfare and the Ministry of Education Science and Culture of Japan.

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