J. Biochem. 120, 133-137 (1996)

The 160k α1(IV) Chain, a Short Form of a Type IV Collagen Polypeptide, of Bovine Lens Capsule Retains the NC1 Domain

Masao Iwata,*[,1] Yoshikazu Sado,[†] Tasuku Sasaki,* Yasutada Imamura,* Yoshifumi Ninomiya,[‡] and Toshihiko Hayashi*[,2]

Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Meguro-ku, Tokyo 153; [†]Division of Immunology, Shigei Medical Research Institute, Yamada, Okayama 701-02; and [‡]Department of Molecular Biology and Biochemistry, Okayama University Medical School, Shikata-cho, Okayama 700

Received for publication, February 22, 1996

We recently reported that the bovine lens capsule contained a shorter α1(IV) chain (160k) as a major polypeptide in addition to the 180k α1(IV) chain [*J. Biochem.* (1995) 117, 1298-1304]. Two experiments were performed to examine whether or not the 160k polypeptide retained the carboxyl-terminal NC1 domain. On immunoblotting analysis with a monoclonal antibody (H11) raised against the NC1 domain of the human α1(IV) chain [positions 1643-1650; near the carboxyl-terminal end of the human α1(IV) chain], the 180k and 160k polypeptides showed identical immunoreactivity, suggesting that the two chains had the same human α1(IV) collagen NC1 domain sequence. Another monoclonal antibody (H21) specific for the NC1 domain of human α2(IV) did not react with these polypeptides, but with the bands corresponding to 175k and 155k. The 160k polypeptide was selectively solubilized from bovine lens capsules, leaving the other major polypeptides, 180k and 175k, insoluble. The 160k polypeptide was separated by preparative electrophoresis. Bacterial collagenase digestion of the separated 160k polypeptide produced collagenase-resistant segments of about 29k and 30k in size based on globular standards. These sizes corresponded well with those of the NC1 domains of type IV collagen α chains (25-30k). The results indicated that the 160k polypeptide retained the carboxyl-terminal NC1 domain of the α1(IV) chain. In turn, the 20k polypeptide of the amino-terminal region or the 7S domain of 180k α1(IV) would have been excised to yield 160k α1(IV), assuming that the 160k α1(IV) chain is a processed form of the 180k α1(IV) one and not an alternatively spliced chain of the α1(IV) gene.

Key words: α1(IV) collagen, basement membrane, bovine lens capsule, collagen IV, noncollagenous domain.

A type IV collagen molecule is, like other collagenous molecules, composed of three polypeptides or α chains. Such protein molecules are characterized by three distinct domains, each comprising three homologous sequences in the polypeptide; an amino-terminal short triple-helical domain (7S domain), the main triple-helical collagenous domain (Gly-Xaa-Yaa triplets) with many interruptions, and a carboxyl-terminal noncollagenous domain (NC1 domain) (*1, 2*). Type IV collagen molecules form a complex three-dimensional meshwork of branched filaments. The meshwork is thought to be formed through a variety of intermolecular interactions. Studies on the aggregation *in vitro* of type IV collagen molecules isolated from mouse EHS tumors suggested that the intermolecular interactions of type IV collagen molecules include the formation of tetramers through 7S domains, the formation of dimers through NC1 domains, lateral interactions between molecules along the triple-helical domains, and NC1 domain-triple helical domain interactions (*3-6*). These interactions involve reversible interactions as well as covalent cross-links (*7, 8*). The type IV collagen network model proposed by Timpl *et al.* (*9*) involves the combination of a tetrameric association at 7S domains and a dimeric association at NC1 domains, with a pore size of the distance between branching points of 800 nm. For another model which includes lateral associations at triple-helical domains in addition, proposed by Yurchenco and Furthmayr (*10*), the pore size would be smaller, *i.e.* about 200 nm. Aggregates reconstituted from an acid extract of bovine lens capsules or from a pepsin-solubilized type IV collagen fraction from human placenta showed polygonal meshworks with distances between branching points of about 15 nm at the maximal frequency (Adachi, E. *et al.*, submitted for publication). Most recently, Muraoka *et al.* (*11*) and Nakazato, K. *et al.* (manuscript in preparation) demonstrated that acid soluble type IV collagen from bovine lens capsules reassembled to form gels. The gels appeared as filamentous meshwork in electron micrographs.

The bovine lens capsule contains two different sizes of α1(IV) collagen chain, 180k and 160k, as major constitutive polypeptides (*12*). The 160k polypeptide is incorpo-

[1] Present address: Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Ibaraki 305.
[2] To whom correspondence should be addressed.
Abbreviations: BPB, bromophenol blue; EHS, Engelbreth-Holm-Swarm; NC, noncollagenous; PBS, phosphate-buffered saline.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

rated into the type IV collagen meshwork reconstituted. The type IV collagen molecules containing the 160k polypeptide, which is shorter by about 20k than the 180k polypeptide, could differentially contribute to the intermolecular interactions of the type IV collagen, and therefore to the formation and structure of the meshwork. The noncollagenous (NC1) domain of type IV collagen is of particular interest as to the interaction properties of type IV collagen molecules, because this domain exhibits versatile specificity in the interaction, presumably depending on the environmental conditions. The NC1 domains could bind to each other to form end-to-end connections, as well as to the triple-helical domain of another molecule at several sites. The binding of the NC1 domain to the triple-helical domain of type IV collagen was reported to be required for the lateral association of triple-helical domains (*6*).

We examined whether or not the 160k polypeptide retained the NC1 domain by immunoblotting with a specific antibody recognizing the carboxyl-terminal end of the NC1 domain, and by analysis of the peptide size after treatment with bacterial collagenase.

MATERIALS AND METHODS

Materials—The monoclonal antibodies, H11 and H21, were established by a novel method, the rat lymph node method, which was previously reported (*13, 14*). The monoclonal antibody, JK-132, used in this study was obtained from the Shiseido Research Center (Yokohama) (*15*). The following chemicals were purchased: trizma base, diaminobenzidine and phenylmethylsulfonyl fluoride from Sigma Chemical. *N*-Ethylmaleimide from Nacalai Tesque, and sodium azide from Wako Pure Chemical Industries. The horseradish peroxidase-conjugated antibody to rat IgG and horseradish peroxidase-conjugated antibody to mouse IgG were obtained from Cappel and Bio-Rad, respectively.

Preparation of Collagen—Fresh bovine eyes obtained at a local slaughterhouse were brought to the laboratory on ice and immediately dissected, and then the lens capsules were removed. The lens capsules were minced, after washing with 20 mM sodium phosphate, pH 7.2, at 4°C, containing a mixture of protease inhibitors, consisting of 5 mM EDTA, 10 mM *N*-ethylmaleimide, 0.1 mM phenylmethylsulfonyl fluoride, and 0.02% (w/v) sodium azide. The homogenate was suspended at 4°C in 0.5 M acetic acid (10 ml per g wet weight of lens capsule). The solubilized materials were collected and stored at 4°C (acid extract).

SDS-PAGE and Immunoblotting—The acid extract was neutralized with Tris buffer containing SDS (final conc., 1.2%), glycerol (final conc., 5%), and BPB, with β-mercaptoethanol (final conc., 5%). The extract was incubated at 80°C for 5 min and then subjected to SDS-PAGE, which was performed with the buffer system of Laemmli (*16*) on a vertical slab gel (1 mm thick) at a constant current of 20 mA. Immunoblotting was performed as reported previously (*12*). The immunoreactivity was detected by color development after incubation of peroxidase with diaminobenzidine and H_2O_2 in PBS.

Preparative Electrophoresis—Preparative electrophoresis was carried out according to the manufacturer's instructions for the apparatus (Nihon Eido; model NA-1800). The supernatant after centrifugation of the extract obtained on incubation of the lens capsule homogenate with PBS at 47°C for 1 h was diluted to Tris-HCl, pH 6.8, containing 1.2% SDS, 5% glycerol, 5% β-mercaptoethanol, and BPB, and then subjected to preparative electrophoresis after incubation at 80°C for 5 min. The cylindrical running gel with a diameter of 36 mm was prepared from 56 ml of a 5% polyacrylamide solution. The stacking gel was prepared from 8 ml of a 3% polyacrylamide solution. The electrophoresis was performed at 100 V. The fraction collector was set to obtain one fraction every 40 drops.

Bacterial Collagenase Treatment of Type IV Collagen Polypeptides—A portion of the fraction containing the 160k polypeptide was treated with bacterial collagenase (courtesy of Dr. Hata at Tokyo Med. & Dent. Univ.) (*17*) (1 μg/ml) in 50 mM Tris-HCl, pH 7.6, containing 4 mM $CaCl_2$, 1 M NaCl, and 5 mM *N*-ethylmaleimide, and another portion of the fraction was treated in the same way but without the collagenase. The incubation of both portions at 25°C was terminated by the addition of EDTA to a final concentration of 10 mM to inactivate collagenase (*15*). The lack of noncollagenolytic protease activity of the bacterial collagenase used was confirmed, since incubation of the enzyme with the noncollagenous proteins used as molecular standards purchased from Bio-Rad under the same conditions did not change the mobilities or staining of the protein bands (data not shown).

RESULTS

Immunoblotting Analysis—Non-enzymatic extracts of bovine lens capsules were immunoblotted with two anti-α1(IV) monoclonal antibodies; H11 recognizing near the carboxyl-terminal end of the α1(IV) chain (positions 1643-

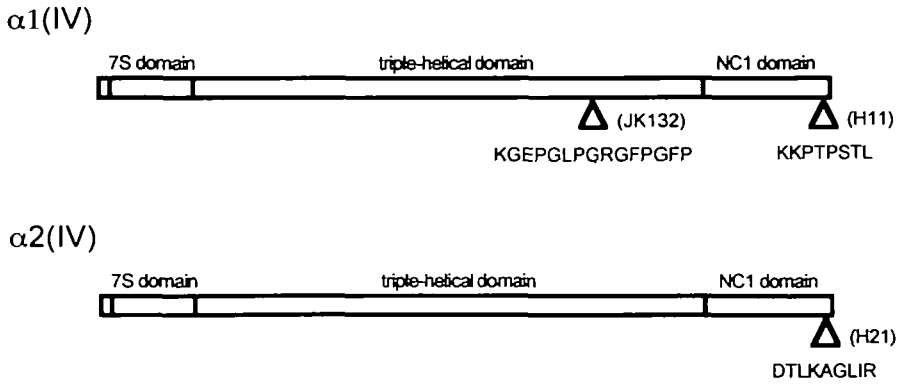

Fig. 1. **Schematic representation of type IV collagen chains and the recognition sites for the monoclonal antibodies.** Monoclonal antibody H11 recognizes the amino acid sequence of 1643-1650 of human α1(IV), and H21 recognizes the amino acid sequence of 1691-1699 of human α2(IV) (*13, 18*). Monoclonal antibody JK-132 recognizes the amino acid sequence contained in the sequence of 1165-1179 of the triple-helical region in the human α1(IV) chain (*12*).

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

1650), and JK-132 recognizing a triple-helical region (positions 1165-1179) (*12, 18*) (Fig. 1). The 180k and 160k polypeptides showed similar relative intensities on immuno-staining, suggesting that the NC1 domain of α1(IV) as well as the triple-helical region was retained in the 160k polypeptide (Fig. 2, panel C). The anti-α2(IV) monoclonal antibody (H21) did not react with these polypeptides, but the bands with slightly higher mobilities, corresponding to M_rs of about 175k and 155k, were intensely stained, suggesting that these two polypeptides were derived from the α2(IV) gene, and possibly that the α2(IV) chain also occurs in two different sizes like the α1(IV) chain (Fig. 2, panels D and E). Another band corresponding to a molecular weight greater than 180k may represent a non-reducibly cross-linked polypeptide of type IV collagen.

Isolation of 160k and 155k Polypeptides—The treatment of a lens capsule homogenate with PBS at 47°C solubilized a portion of the 160k polypeptide, the 180k and 175k polypeptides remaining insoluble. This fraction was further purified by preparative electrophoresis. The 160k polypeptide obtained with this procedure was free from the 180k and 175k polypeptides, as assessed by SDS-PAGE analysis (Fig. 3, lane 3). The 160k polypeptide in the fraction reacted with the monoclonal antibody which specifically recognizes the bovine and human α1(IV) chain at the triple-helical region (JK-132) (Fig. 3, lane 5). The polypeptide also reacted with monoclonal antibody H11, which is specific for the NC1 domain of α1(IV) (Fig. 3, lane 6). The fraction obtained on preparative electrophoresis gave a 155k band that was immunoreactive with the anti-α2(IV) monoclonal antibody (H21) (Fig. 3, lane 7). The 155k band was not obviously stained with amido black (Fig. 3, lane 3). Neither the 180k nor the 175k band was observed on immunoblotting using these monoclonal antibodies, even when the amounts of the samples used were ten times as much as those in lanes 5, 6, and 7, respectively (data not shown).

Bacterial Collagenase Digestion—On SDS-PAGE of the

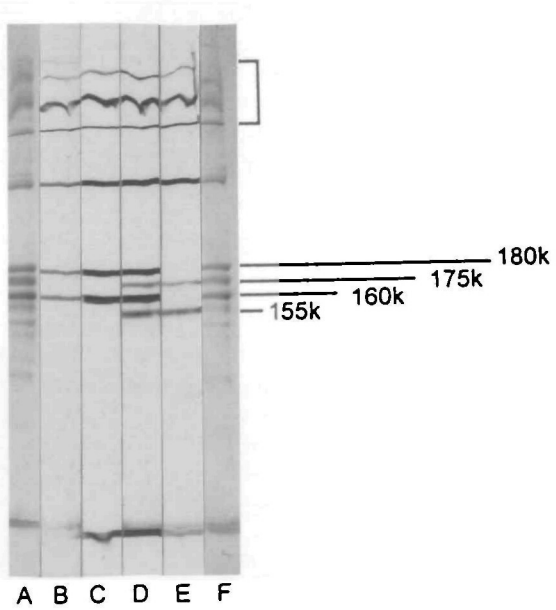

Fig. 2. **Immunoblotting of bovine lens capsule type IV collagen with monoclonal antibodies to NC1 domains.** The extract from bovine lens capsules with 0.5 M acetic acid was resolved on a 5% polyacrylamide gel under reducing conditions. The proteins were electrophoretically transferred to a nitrocellulose membrane, and then strips cut out of the membrane were stained with either amido black or monoclonal antibodies. Panels A and F were stained with amido black. Panel B was stained with the monoclonal antibody that recognizes the triple-helical region of α1(IV) (JK-132). Panel C was stained with anti-α1(IV)NC1 domain (H11). Panel E was stained with anti-α2(IV)NC1 domain (H21). Panel D was stained with a mixture of anti-α1(IV)NC1 and anti-α2(IV)NC1. "]" denotes the stacking gel (3% polyacrylamide gel).

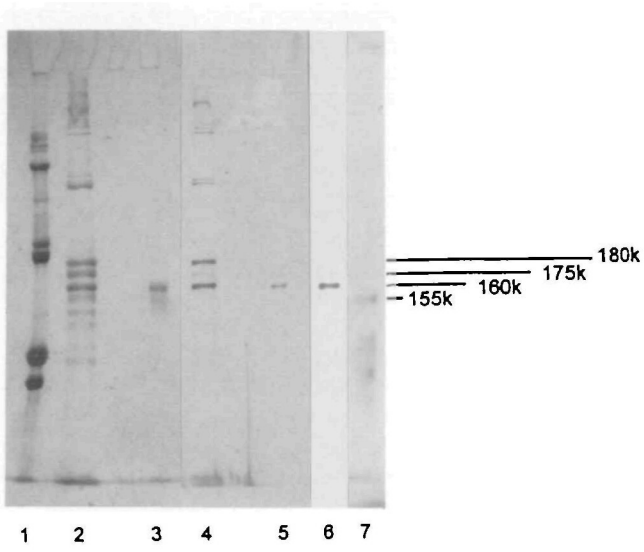

Fig. 3. **Isolation of the 160k and 155k polypeptides by preparative electrophoresis.** Lane 1: type I collagen as a reference protein; lanes 2 and 4: original preparation of bovine lens capsule type IV collagen; lanes 3, 5, 6, and 7: the separated fraction containing 160k polypeptides. Lanes 1, 2, and 3 are amido black stained. Lanes 4 and 5 show immunoblotting with the monoclonal antibody that recognizes the triple-helical region of α1(IV) (JK-132). Lane 6 shows immunoblotting with anti-α1(IV)NC1 domain (H11), and lane 7 that with anti-α2(IV)NC1 domain (H21). The relative amounts of the original type IV collagen and the separated polypeptides in the lanes were changed as follows. 1 and 1/10 in lanes 2 and 4; and 1, 1/10, 1/10, and 3/10 in lanes 3, 5, 6, and 7.

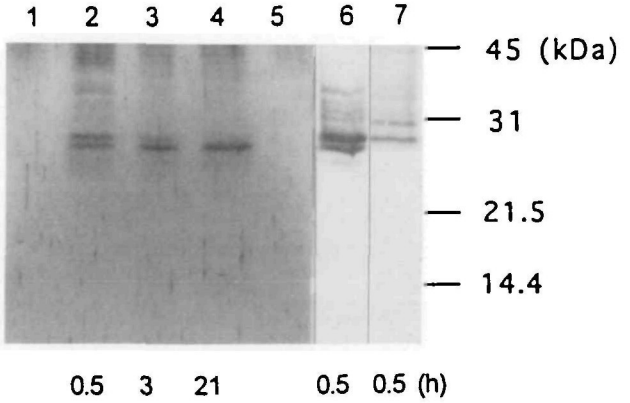

Fig. 4. **SDS-PAGE and immunoblotting analyses of bacterial collagenase-resistant fragments of 160k and 155k polypeptides.** Lane 1: the fraction containing the 160k polypeptide; lanes 2, 3, 4, 6, and 7: fragments obtained on bacterial collagenase treatment of the fraction for the indicated times in h; lane 5: bacterial collagenase alone. Lanes 1-5 were silver-stained. Lanes 6 and 7 were immunostained with H11 and H21, respectively. All the samples were reduced prior to electrophoresis.

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

separated 160k polypeptide after treatment with bacterial collagenase at 25°C for 0.5 h, two bands were observed, corresponding to apparent molecular weights of 29k and 30k based on globular protein standards (Fig. 4, lane 2). These bands were immunostained with H11, while the 30k band was also immunostained with H21 (Fig. 4, lanes 6 and 7), demonstrating that the protein bands contained the α1(IV) and α2(IV) NC1 polypeptides. Previous studies revealed that collagenase-resistant segments (25–30k) of type IV collagen were similar in size, regardless of the α chains, to separated polypeptides of NC1 domains (*19, 20*). This is consistent with the presence of the α1(IV) NC1 domain within the 160k [α1(IV)] polypeptide and the α2(IV) NC1 domain within the 155k [α2(IV)] polypeptide.

DISCUSSION

EHS tumor type IV collagen is composed of two α size polypeptides corresponding to the α1 and α2 chains (*21*), while bovine lens capsule type IV collagen contains three α size polypeptides (M_r = 180k, 175k, and 160k) as major components (*22*). Among the three polypeptides in the lens capsule, the 180k and 160k ones were shown to originate from the same gene of the α1(IV) chain (*12*). The amount of the 160k polypeptide was as great as that of the 180k polypeptide in both the non-enzymatic extract with 0.5 M acetic acid at 4°C and the extract with neutral buffer containing SDS and DTT at 80°C. The latter extracting conditions solubilized essentially all the polypeptides comprising the bovine lens capsule. The question then arose as to which portion of the 180k α1(IV) polypeptide, of 20k in size, is missing from the 160k α1(IV) chain in the bovine lens capsule. The present study addressed the question of whether the 160k polypeptide possesses the NC1 domain or not. Two examinations were carried out; whether or not the 160k α1(IV) chain reacts with the monoclonal antibody specifically recognizing the carboxyl-terminal end of the α1(IV) NC1 domain, and whether or not the chain retains a noncollagenous domain with a size corresponding to that of the NC1 domain of type IV collagen α chains after treatment with bacterial collagenase. The results indicated that the 160k polypeptide retains the NC1 domain of the α1(IV) chain intact.

Type I collagen is synthesized and secreted from cells as a precursor form, type I procollagen, with non-helical domains at either end. In the process of self-assembly or fibrillogenesis of type I collagen, the propeptides at the ends are cleaved (extracellularly) by specific N- and C-proteinases. On the other hand, the processing of type IV collagen molecules has not been documented, and it has been believed that type IV collagen synthesized is deposited on a cell layer as a secreted form. Taylor and Grant (*23*) reported that the 160k polypeptide was only found in tissue extracts, *i.e.* not in culture media, of adult bovine lens capsular epithelia in organ culture, but that 180k and 175k polypeptides were present both in media and tissue extracts of bovine lens capsule organ cultures. In the case of a human fetal lung fibroblast culture, the cell layer deposit gave two immunoreactive bands at the positions of 180k and 160k with the anti-α1(IV) monoclonal antibody, while the culture medium only gave a 180k immunoreactive band (Sasaki, T., Takahashi, S. *et al.*, unpublished data). Our preliminary data showed that only one major α1(IV)-mRNA was detected in the cells (data not shown). Thus, these studies suggested that the 160k polypeptide was likely to be synthesized initially as 180k and secreted into the medium, and then a portion of the 180k polypeptide would be processed to the 160k form during or after deposition on the cell layer. Then, if the cell layer 160k α1(IV) chain is essentially the same as bovine lens capsule 160k α1(IV), which, as the present study demonstrated, retains the intact NC1 domain, the 180k polypeptide in culture media should be processed to the 160k polypeptide with the carboxyl-terminal region intact, suggesting indirectly that the processing would comprise the removal of the 7S region of the 180k polypeptide.

Furthermore, if the 160k polypeptide α1(IV) lacks the 7S domain consisting of about 220 residues (*24*), a significant amount of type IV collagen molecules with the chain composition, $[\alpha 1(IV)]_2\alpha 2(IV)$, should contain one or two 160k polypeptides, suggesting that these molecules containing 160k α1(IV) would eventually show different interaction properties through the 7S domains. Since it is well known that type IV collagen tetramers are covalently bonded through their 7S domains, dissociation of the 160k polypeptide from the extract of bovine lens capsules under milder conditions than for the 180k and 175k polypeptides (Muraoka, M. *et al.*, unpublished data) may be relevant as to the lack of the amino-terminal 7S domain in the 160k polypeptide.

The finding that two bands were immunoblotted with the anti-α2(IV) monoclonal antibody suggests that they may represent counterparts of the 180k and 160k α1(IV) chains which retain the α1(IV) NC1 domain. Molecules containing two 160k α1(IV) polypeptides and one truncated α2(IV) are theoretically possible. These molecules could be involved in the formation of the NC1 dimer, the NC1 to triple-helical interaction, and the inter-triple helical lateral associations, but be free from the restraint of the tetramer association through 7S domains. Thus, such truncated type IV collagen molecules may contribute to loosening of the architecture or increased flexibility of the type IV collagen network.

Short forms of α1(IV) and α2(IV) chains were also demonstrated by the immunoreactivity to the monoclonal antibodies (Sasaki, T. *et al.*, manuscript in preparation) in non-enzymatic tissue extracts from human placenta and bovine kidney.

REFERENCES

1. Kühn, K. (1995) Basement membrane (Type IV) collagen. *Matrix Biol.* **14**, 439–445
2. Hudson, B.G., Reeders, S.T., and Tryggvason, K. (1993) Type IV collagen: Structure, gene organization, and role in human diseases. *J. Biol. Chem.* **268**, 26033–26036
3. Siebold, B., Qian, R.A., Glanville, R.W., Hofmann, H., Deutzmann, R., and Kühn, K. (1987) Construction of a model for the aggregation and cross-linking region (7S domain) of type IV collagen based upon an evaluation of the primary structures of the α1 and α2 chains in this region. *Eur. J. Biochem.* **168**, 569–575
4. Yurchenco, P.D. and Ruben, G.C. (1988) Type IV collagen lateral association in the EHS tumor matrix. *Am. J. Pathol.* **132**, 278–291
5. Leblond, C.P. and Inoue, S. (1989) Structure, composition, and assembly of the basement membrane. *Am. J. Anat.* **185**, 367–390
6. Tsilibary, E.C. and Charonis, A.S. (1986) The role of the main

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012

noncollagenous domain (NC1) in type IV collagen self-assembly. *J. Cell Biol.* **103**, 2467-2473
7. Weber, S., Dolz, R., Timpl, R., Fessler, J.H., and Engel, J. (1988) Reductive cleavage and reformation of the interchain and intrachain disulfide bonds in the globular hexameric domain, NC1, involved in network assembly of basement membrane collagen (type IV). *Eur. J. Biochem.* **175**, 229-236
8. Ries, A., Engel, J., Lustig, A., and Kühn, K. (1995) The function of the NC1 domains in type IV collagen. *J. Biol. Chem.* **270**, 23790-23794
9. Timpl, R., Wiedemann, H., Van Delden, V., Furthmayr, H., and Kühn, K. (1981) A network model for the organization of type IV collagen molecules in basement membranes. *Eur. J. Biochem.* **120**, 203-211
10. Yurchenco, P.D. and Furthmayr, H. (1984) Self-assembly of basement membrane collagen. *Biochemistry* **23**, 1839-1850
11. Muraoka, M., Nakazato, K., and Hayashi, T. (1996) Gel formation from the Type IV collagen isolated from bovine lens capsule in guanidine-HCl and dithiothreitol. *J. Biochem.* **119**, 167-172
12. Iwata, M., Imamura, Y., Sasaki, T., and Hayashi, T. (1995) Evidence for a short form of α1(IV) as a major polypeptide in bovine lens capsule. *J. Biochem.* **117**, 1298-1304
13. Sado, Y., Kagawa, M., Kishiro, Y., Sugihara, K., Naito, I., Seyer, J.M., Sugimoto, M., Oohashi, T., and Ninomiya, Y. (1995) Establishment by the rat lymph node method of epitope-defined monoclonal antibodies recognizing the six different α chains of human type IV collagen. *Histochem. Cell Biol.* **104**, 267-275
14. Kishiro, Y., Kagawa, M., Naito, I., and Sado, Y. (1995) A novel method of preparing rat-monoclonal antibody-producing hybridomas by using rat medial iliac lymph node cells. *Cell Struct. Funct.* **20**, 151-156
15. Kino, J., Adachi, E., Yoshida, T., Nakajima, K., and Hayashi, T. (1988) Characterization of a monoclonal antibody against human placenta type IV collagen by immunoelectroblotting, antibody-coupled affinity chromatography, and immunohistochemical localization. *J. Biochem.* **103**, 829-835
16. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **27**, 680-685
17. Hata, R., Ninomiya, Y., Nagai, Y., and Tsukada, Y. (1980) Biosynthesis of interstitial types of collagen by albumin-producing rat liver parenchymal cell (Hepatocyte) clones in culture. *Biochemistry* **19**, 169-176
18. Hostikka, S.L. and Tryggvason, K. (1988) The complete primary structure of the α2 chain of human type IV collagen and comparison with the α1(IV) chain. *J. Biol. Chem.* **263**, 19488-19493
19. Wieslander, J., Barr, J.F., Butkowski, R.J., Edwards, S.J., Bygren, P., Heinegard, D., and Hudson, B.G. (1984) Goodpasture antigen of the glomerular basement membrane: Localization to noncollagenous regions of type IV collagen. *Proc. Natl. Acad. Sci. USA* **81**, 3838-3842
20. Langeveld, J.P., Wieslander, J., Timoneda, J., McKinney, P., Butkowski, R.J., Wisdom, B.J., Jr., and Hudson, B.G. (1988) Structural heterogeneity of the noncollagenous domain of basement membrane collagen. *J. Biol. Chem.* **263**, 10481-10488
21. Kleinman, H.K., McGarvey, M.L., Liotta, L.A., Rubey, P.G., Tryggvason, K., and Martin, G.R. (1982) Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from the EHS sarcoma. *Biochemistry* **21**, 6188-6193
22. Muraoka, M. and Hayashi, T. (1993) Three polypeptides with distinct biochemical properties are major α chain-size components of type IV collagen in bovine lens capsule. *J. Biochem.* **114**, 358-362
23. Taylor, C.M. and Grant, M.E. (1985) Assembly of chick and bovine lens-capsule collagen. *Biochem. J.* **226**, 527-536
24. Glanville, R.W., Qian, R.Q., Siebold, B., Risteli, J., and Kühn, K. (1985) Amino acid sequence of the N-terminal aggregation and cross-linking region (7S domain) of the α1(IV) chain of human basement membrane collagen. *Eur. J. Biochem.* **152**, 213-219

Downloaded from http://jb.oxfordjournals.org/ at Changhua Christian Hospital on October 2, 2012