doi: 10.1111/j.1346-8138.2006.00127.x

ORIGINAL ARTICLE

Interactions between epiplakin and intermediate filaments

Wenqing WANG,[1]* Hideaki SUMIYOSHI,[2] Hidekatsu YOSHIOKA,[2] Sakuhei FUJIWARA[1]

Department of Anatomy, Biology and Medicine ([1]Dermatology and [2]Biochemistry), Faculty of Medicine, Oita University, Hasama-machi, Yufu, Japan

ABSTRACT

Epiplakin, a cytoskeletal linker protein, was originally identified as an autoantigen in a serum specimen obtained from a patient with subepidermal blistering disease. To examine the binding ability of epiplakin with intermediate filaments (IF), we performed slot-blot assays using fusion proteins that included various domains and subdomains of epiplakin. At least two of the 4.6 copies in the B domains of epiplakin were necessary for the binding of fusion proteins to keratin. The repeated structures of linker domains also played an important role in the binding of epiplakin to keratin in these assays while also increasing the repeated structure in the linker domain of epiplakin which is involved in the increased binding to IF. A similar but weaker binding to vimentin and desmin was also detected. These observations indicated that the highly repeated structures of epiplakin in both the B and the linker domains, which is the unique feature of this molecule in the plakin family, play an essential role in the functioning of this molecule.

Key words: autoantigen, epidermis, epiplakin, intermediate filaments, plakin.

INTRODUCTION

Epiplakin, a cytoskeletal linker protein, was originally identified as an autoantigen in a serum specimen obtained from a patient with subepidermal blistering disease. Epiplakin is expressed in the stratified epithelium and also in the simple epithelium.[1,2] It belongs to the plakin family and the human and murine forms have molecular masses of 552 kDa and 725 kDa, respectively. The human and murine forms also include 13 domains and 16 domains, respectively, which are homologous to the B domain in the carboxy-terminal region of desmoplakin. The five carboxy-terminal B domains in human epiplakin, together with their associated linker regions, are strongly conserved.

Research on the functions of epiplakin is now underway. *In vivo*, epiplakin is co-expressed with keratin 6 in the epidermis during wound healing.[3] As in other members of the plakin family, for example, desmoplakin, BPAG1 and plectin, each B domain, as well as the A and C domains in the carboxy-terminal region of desmoplakin, contains 4.6 copies per unit of a 38-amino-acid motif, which is known as the plakin repeat domain (PRD).[4,5] Both plectin and desmoplakin have been shown to interact directly with intermediate filaments (IF) through their carboxy terminus containing PRD.[6,7] A recent study, using a co-sedimentation assay, showed that the B and C domains of desmoplakin interact with vimentin and, moreover, the crystal structure of each domain reveals a globular structure, with a unique fold, with which vimentin interacts.[8] In addition to the PRD, linker subdomains that are found before the C domain or after the rod domain of all conventional plakins, such as periplakin, envoplakin, desmoplakin, BPAG1 and plectin but not epiplakin, are involved in interactions with IF.[9] Another study showed the linker region of desmoplakin to be associated with vimentin and simple epithelial keratin.[10]

In a recent series of *in vitro* experiments, Jang *et al.* showed the B domain or the linker plus the B

*Present address: Department of Dermatology, the Fourth Affiliated Hospital of Hebei Medical University, Shijiazhuang 050011, China.
Correspondence: Sakuhei Fujiwara, MD, PhD, Department of Anatomy, Biology and Medicine (Dermatology), Oita University, Idaigaoka 1-1, Hasama-machi, Yufu 879-5593, Japan. Email: fujiwara@med.oita-u.ac.jp
Received 24 November 2005; accepted 25 January 2006.

domain of human epiplakin, but not the linker domain alone, to interact with keratin and vimentin.[11] In "knock-down and rescue" experiments using HeLa cells, they showed the linker plus the B domain fragment, but neither the B nor the linker, to be able to restore the collapsed keratin and vimentin IF network.

According to these previously published observations, it seems likely that the function of epiplakin involves association with IF. We focused on the highly repeated structures of both the B and the linker domains of epiplakin[2] and we attempted to clarify the function of the repeated structure of this molecule while addressing the following questions. Do epiplakin domains actually bind to IF? If so, which domain(s) and how many repeats in the domain(s) of epiplakin are essential for binding and with which IF does this domain(s) interact most strongly?

We herein report our recent findings that all three domains of epiplakin, namely the B, linker, and the B domain plus the linker domains (referred to here as the B+linker domain), interact with IF, and that the repeated structures of these domains play an important role in the interactions of epiplakin with keratin using slot-blot assays.

MATERIALS AND METHODS

Construction of plasmids for the expression of GST-B, GST-linker, GST-B+linker, PRD in the B domain, and mutated linker domains

In order to generate fusion proteins that consisted of glutathione S-transferase (GST) and a B domain (B8), GST and a linker domain (L8-9) and GST and a B+linker domain (B8+L8-9), as well as fusion proteins with PRD in the B domain, and truncated linker domains, we performed polymerase chain reaction (PCR) using a 17-kbp fragment of the human gene for epiplakin (NT_2) as a template (Fig. 1).[12] As a result, we chose domain B8, because the sequence of this domain is the same as that of five B domains in the downstream region of this macromolecule. To express fusion proteins with PRD in the B domain, we made GST-fusion proteins that included one, two and three copies of the PRD (1R-3R). To express the fusion proteins with linker repeats, we constructed plasmids that included one, two and three repeats of the linker domain (L1R-L3R; Fig. 1). The primers that we used are shown in Table 1. All products of PCR were subcloned into the PGEM T-Easy vector (Promega, Madison, WI, USA) and then were inserted into the PGEX 4T-1, PGEX 4T-2, or

Table 1. Primers Combinations

Molecule	Primers	Length (bp)
B domain		
Sense	5′-CGCAGGAGAAGATGAGCATC-3′	528
Antisense	5′-CAGCATGTAGAGCCCGCTGT-3′	
Linker		
Sense	5′-CAGCTGGCAGGCCGGGGCTC-3′	1074
Antisense	5′-GCGGCCGGGCTGGTCCTTGG-3′	
B+Linker		
Sense	5′-AACGGGACGGAGCACGACGCAA-3′	1717
Antisense	5′-GCGGCCGGGCTGGTCCTTGG-3′	
1R		
Sense	5′-GGAATTCCAGGAGAAGATGAGCATCTA-3′	114
Antisense	5′-GCTCGAGGCGCACGGGGTCGATGACGA-3′	
2R		
Sense	5′-GGAATTCCAGGAGAAGATGAGCATCTA-3′	228
Antisense	5′-GCTCGAGGGTGTAGGGGTCGGTGTAGC-3′	
3R		
Sense	5′-GGAATTCCAGGAGAAGATGAGCATCTA-3′	342
Antisense	5′-GCTCGAGGTGCACGGGGTCGATGAC-3′	
L1R		
Sense	5′–GGAATTCCAGCTGGCAGGCCGGGGC-3′	246
Antisense	5′-GCTCGAGCTGGGCCTGGGCCAGCAGCG-3′	
L2R		
Sense	5′–GGAATTCCAGCTGGCAGGCCGGGGC-3′	492
Antisense	5′-GCTCGAGCTCGGCCTCCTCGATGATGG-3′	
L3R		
Sense	5′–GGAATTCCAGCTGGCAGGCCGGGGC-3′	855
Antisense	5′-GCTCGAGCTCCGTCTCCTCGATGACCC-3′	

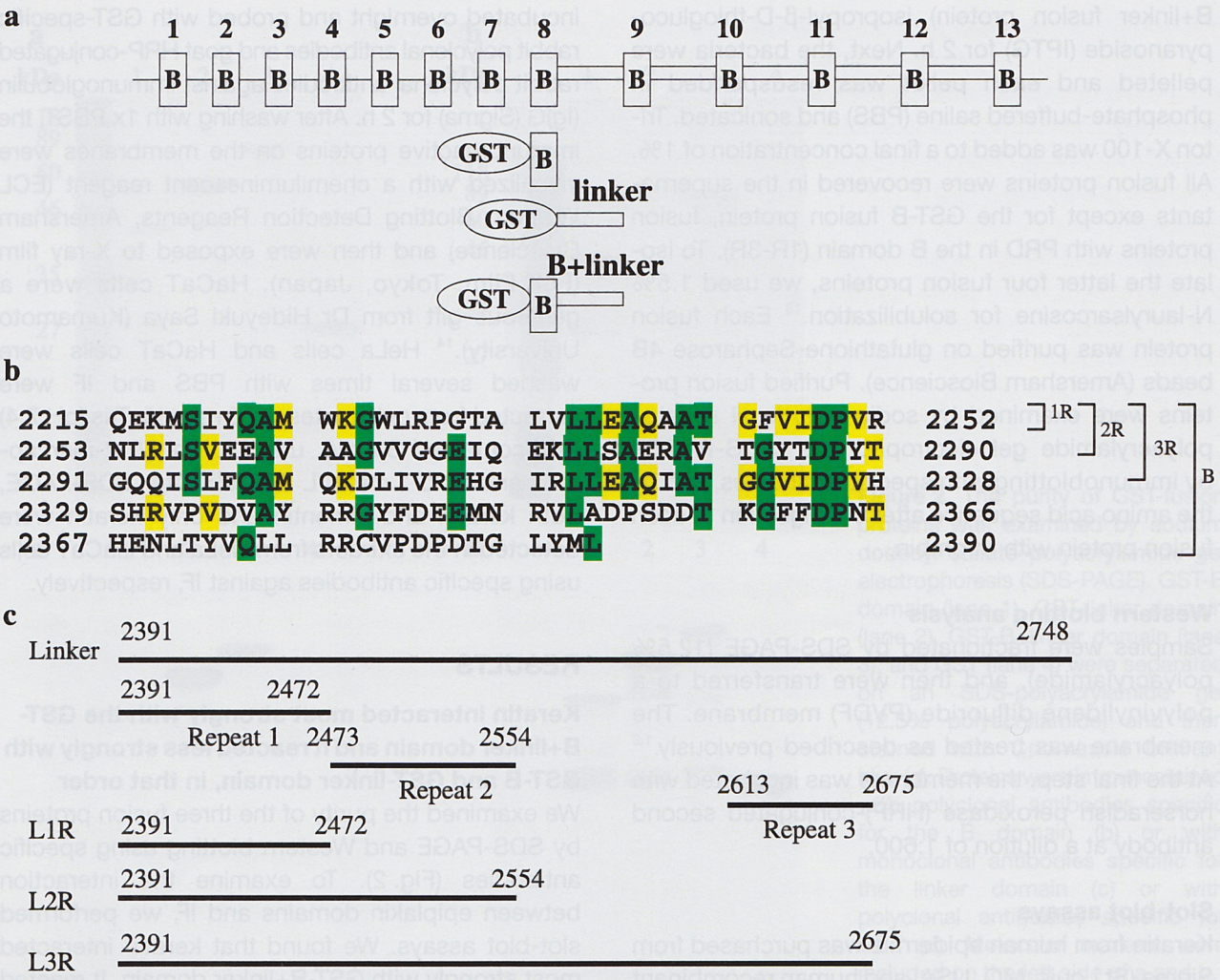

Figure 1. Schematic representation of epiplakin (a) and the repeat structures of the B domain (b) and the linker domain (c). (a) Numbered boxes indicate B domains that are 46–70% homologous to the B domain in desmoplakin. The double-lined linker regions and the five corresponding B domains (9–13) are almost identical.[2] We performed polymerase chain reaction (PCR) to generate glutathione S-transferase (GST)-B domain (8B), GST-linker domain (L8-9), and GST-B+linker domain (B8+L8-9). (b) The structure of each B domain includes 4.6 copies of a motif of 38 amino acids, which is referred to as the plakin repeat domain (PRD). The sequence shown is that of B8. When at least three amino acid residues are identical in the 4.6 copies, they are shown in green, while identical amino acid residues in the first and third or second and fourth copies are shown in yellow. 1R denotes a GST-fusion protein that contains one copy unit, and 2R and 3R denote fusion proteins with two and three copies units, respectively. (c) The structure of the linker domain between B8 and B9, with 2.7 repeats of a sequence of 82 amino acids.[2] These repeats are lined up along the entire linker domain. L1R denotes the GST-fusion protein that included the first one repeat of the linker domain (repeat 1). L2R and L3R denote GST-fusion proteins that included two linker repeats (repeat1+repeat2) and three linker repeats (repeat1-3), respectively. L3R also included a non-repeated structure (residue 2555-2612).

PGEX 4T-3 vector (Amersham Bioscience, Piscataway, NJ, USA), as described previously.[2]

Expression and purification of GST-fusion proteins

Glutathione S-transferase-fusion proteins were expressed in *Escherichia coli* and purified as described previously.[2] In brief, *E. coli* DH5α cells, into which plasmids that encoded the fusion proteins had been introduced, were grown at 37°C (or 32°C for the GST-B fusion protein) and were treated with by 0.2 mmol/L (or 0.1 mmol/L for the GST-

B+linker fusion protein) isopropyl-β-D-thiogluco-pyranoside (IPTG) for 2 h. Next, the bacteria were pelleted and each pellet was resuspended in phosphate-buffered saline (PBS) and sonicated. Triton X-100 was added to a final concentration of 1%. All fusion proteins were recovered in the supernatants except for the GST-B fusion protein, fusion proteins with PRD in the B domain (1R-3R). To isolate the latter four fusion proteins, we used 1.5% N-laurylsarcosine for solubilization.[13] Each fusion protein was purified on glutathione-Sepharose 4B beads (Amersham Bioscience). Purified fusion proteins were examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), by immunoblotting with specific antibodies, and by the amino acid sequence after the digestion of each fusion protein with thrombin.

Western blotting analysis

Samples were fractionated by SDS-PAGE (12.5% polyacrylamide), and then were transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was treated as described previously.[1,2] At the final step, the membrane was incubated with horseradish peroxidase (HRP)-conjugated second antibody at a dilution of 1:600.

Slot-blot assays

Keratin from human epidermis was purchased from Sigma (St Louis, MO, USA) and human recombinant vimentin and desmin were purchased from Progen (Heidelberg, Germany). All IF were polymeric. Keratin was solubilized in 20 mmol/L Tris/HCl buffer pH 7.5 containing 3.2 mol/L urea, 0.04 mol/L β-mercaptoethanol. Vimentin and desmin were solubilized in 12 mmol/L Tris/HCl buffer pH 8.0 containing 3.8 mmol/L urea, 0.8 mmol/L dithiothreitol, 0.8 mmol/L ethylene diamine tetra acetate (EDTA) and 4 mmol/L methylammonium chloride and they were immobilized on a PVDF membrane using a Bio-Dot microfiltration apparatus (Bio-Rad, Hercules, CA, USA). After blocking with 4% nonfat milk in 1x PBST (PBS that contained 0.1% Tween 20), each piece of membrane was put in a hybridization bag with the indicated fusion protein (GST-B, 1.0 μg/ml; GST-linker, 1.3 μg/ml; GST-B+linker, 1.6 μg/ml; these concentrations are equal on a molar basis) in blocking solution (4% nonfat milk in PBST) and then it was incubated overnight and probed with GST-specific rabbit polyclonal antibodies and goat HRP-conjugated rabbit polyclonal antibodies against immunoglobulin (Ig)G (Sigma) for 2 h. After washing with 1x PBST, the immunoreactive proteins on the membranes were visualized with a chemiluminescent reagent (ECL Western Blotting Detection Reagents, Amersham Bioscience) and then were exposed to X-ray film (Fuji Film, Tokyo, Japan). HaCaT cells were a generous gift from Dr Hideyuki Saya (Kumamoto University).[14] HeLa cells and HaCaT cells were washed several times with PBS and IF were extracted from cell lysates in 25 mmol/L Tris (pH 7.4) that contained 8 mol/L urea, 0.1 mol/L 2-mercaptoethanol and 1 mmol/L EDTA.[15] After SDS-PAGE, both keratin and vimentin and only keratin were detected in the extracts from HeLa and HaCaT cells using specific antibodies against IF, respectively.

RESULTS

Keratin interacted most strongly with the GST-B+linker domain and it reacted less strongly with GST-B and GST-linker domain, in that order

We examined the purity of the three fusion proteins by SDS-PAGE and Western blotting using specific antibodies (Fig. 2). To examine the interaction between epiplakin domains and IF, we performed slot-blot assays. We found that keratin interacted most strongly with GST-B+linker domain. It reacted less strongly with GST-B and GST-linker domain, in that order (Fig. 3). A low concentration (1.3 μg/ml) of GST-linker reacted with keratin very weakly, however, a high concentration (3 μg/ml) of this protein was found to significantly react with keratin as described later (Fig. 6). Although vimentin and desmin also bound to GST-B+linker, GST-B, and GST-linker domain, in a descending order of avidity, the binding was weaker than that to keratin. These data on the interactions of GST-B+linker with keratin and vimentin are essentially similar to the data reported by Jang *et al.*[11] According to GST-B, there is some discrepancy with their data which will be discussed later. However, our finding that the linker domain interacts with IF and that each domain interacts more strongly with keratin than with vimentin or desmin is a new discovery. Such interactions might explain the higher affinity for IF of B+linker than of

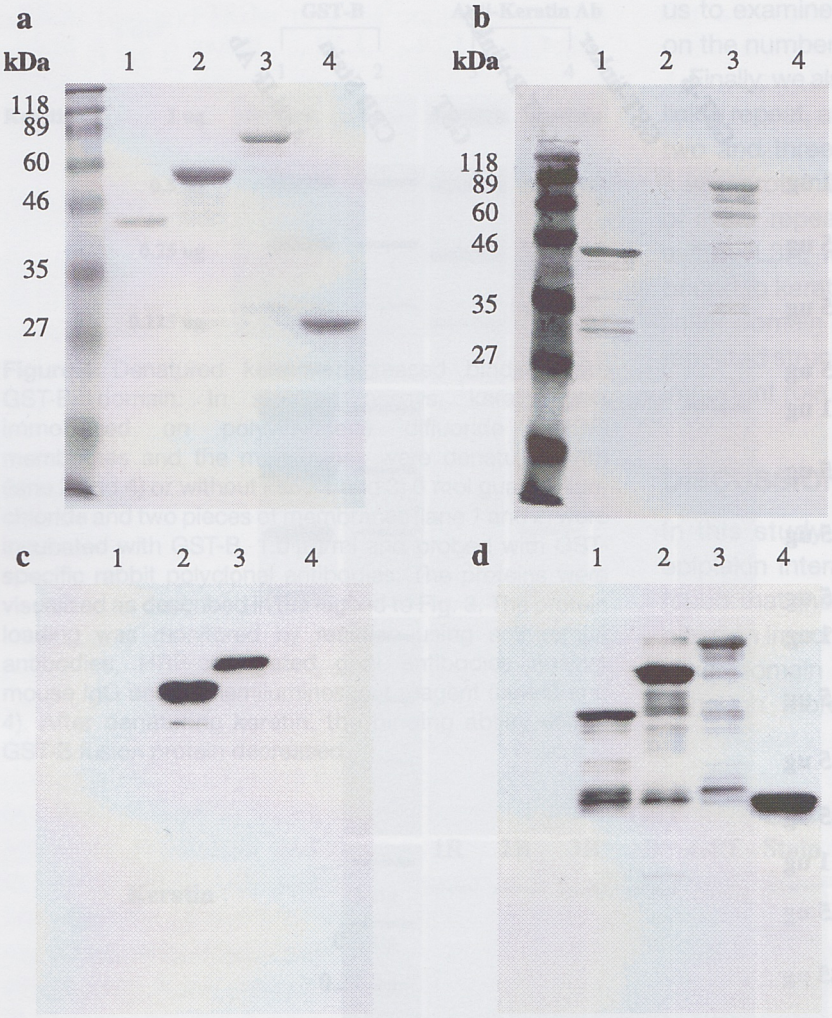

Figure 2. The purity of GST-fusion proteins was examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). GST-B domain (lane 1), GST-linker domain (lane 2), GST-B+linker domain (lane 3), and GST (lane 4) were separated on an SDS-polyacrylamide gel (12.5% polyacrylamide) and then stained with Coomassie brilliant blue (a). Proteins were immunostained with polyclonal antibodies specific for the B domain (b) or with monoclonal antibodies specific for the linker domain (c) or with polyclonal antibodies specific for GST (d). Molecular markers were included on the left side of a and b.

the B domains alone. This discrepancy, in terms of the binding properties of the linker domain between this and the report by Jang *et al.*, might be due to the instability of the binding of the GST-linker fusion protein. We found that the freshly prepared GST-linker fusion protein had reproducible binding activity. When IF were denatured on the blotting membrane by treatment with 6 mol guanidinium chloride, the extent of the interactions with each domain decreased (Fig. 4). These data suggest that the higher-order structure of IF is necessary for their interactions with the various domains of epiplakin.

Repeated structures of the B and linker domains are important for the binding of epiplakin to keratin

We attempted next to identify the structures in the B and linker domains that are important for the binding of epiplakin to keratin. We first analyzed the interactions of keratin with several deletion mutants of the GST-B fusion protein. Each mutant protein lacked one-quarter of the entire B domain but each still interacted strongly with IF, suggesting that the repeated structure of the B domain plays an important role in the interaction with IF (data not shown). We next performed experiments for which the results are shown in Figure 5. At least two (2R), and almost three (3R) copies of the 4.6 copies in the B domains were necessary for their binding to keratin. When we used IF extracted from HeLa or HaCaT cells instead of purified keratin, we obtained the same results (Fig. 5).

The linker domain also includes three repeats of a motif and critical amino acids are conserved in human and mouse linker domains.[2,16] In a preliminary study, similar to the experiment of the B

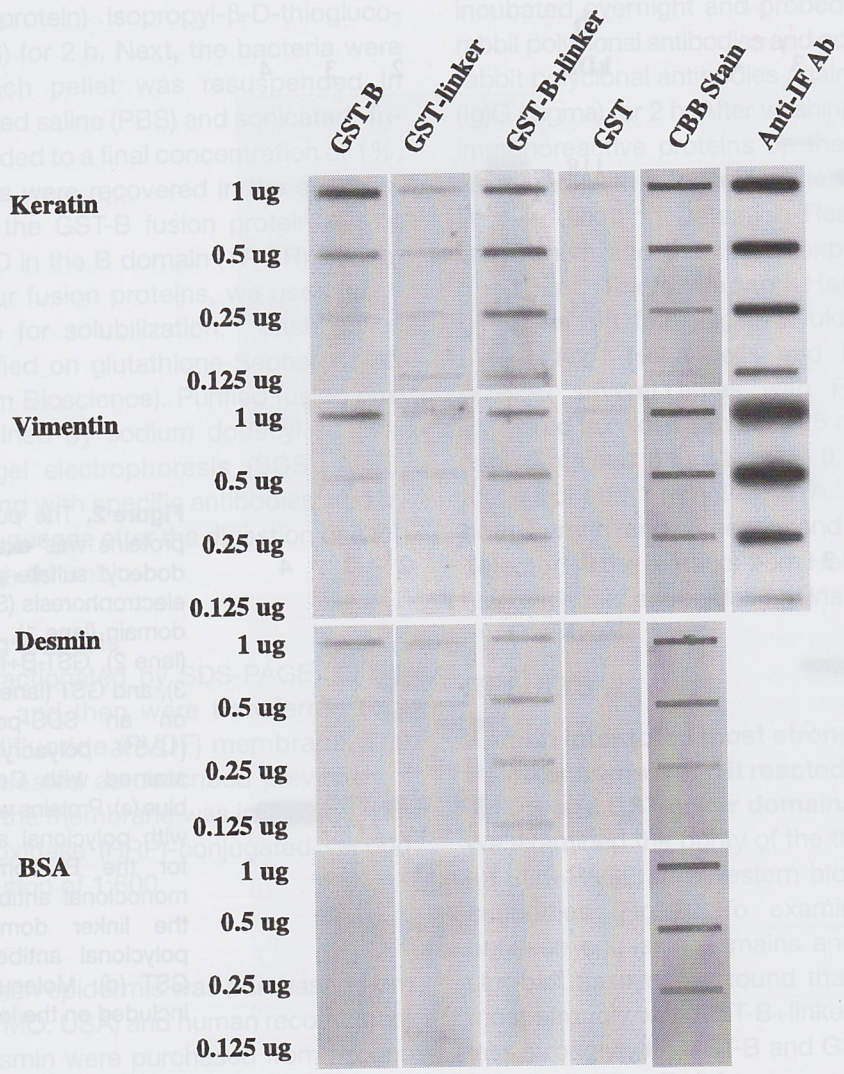

Figure 3. Keratin interacted most strongly with GST-B+linker domain, less strongly with GST-B and least strongly with GST-linker domain. In slot-blot assays, the indicated protein or bovine serum albumin (BSA; as a control) was immobilized on membranes and then the membranes were incubated with the indicated fusion protein (GST-B, 1.0 μg/ml; GST-linker, 1.3 μg/ml; GST-B+linker, 1.6 μg/ml; these concentrations are equal on a molar basis.) or with GST (as a control) and probed with GST-specific rabbit polyclonal antibodies and horseradish peroxidase (HRP)-conjugated goat antibodies against rabbit immunoglobulin (Ig)G. The immunoreactive proteins on membranes were visualized by chemiluminescence and exposure to X-ray film. The protein loading was monitored with Coomassie brilliant blue staining (CBB) or reaction using anti-keratin (right upper panel) or anti-vimentin (right lower panel) antibodies and HRP-conjugated goat antibodies against mouse IgG. The immunoreactive proteins on membranes were visualized described above. Keratin interacted most strongly with GST-B+linker domain and it reacted less strongly with GST-B and GST-linker domain, in that order. Although vimentin and desmin also bound to GST-B+linker, GST-B, and GST-linker domain, in a descending order of avidity, the binding was weaker than that to keratin.

domain, we made fusion proteins with four deletion mutants of the linker domain, each of which lacked one-fourth of the entire domain. In a slot-blot assay, one deletion mutant, which lacked only 18 amino acid residues of the third linker repeat, bound to keratin as strongly as did the entire linker domain, while the other three deletion mutants, which lacked the longer part of the first, second and third linker repeat, either failed to bind or did bind to keratin weakly (data not shown). This observation tempted

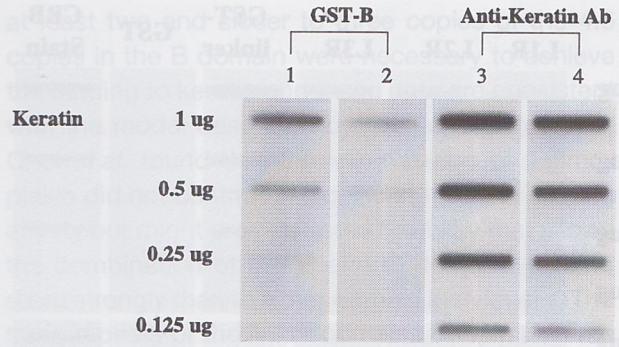

Figure 4. Denatured keratin decreased binding with GST-B domain. In slot-blot assays, keratin was immobilized on polyvinylidene difluoride (PVDF) membranes and the membranes were denatured with (lane 2 and 4) or without (lane 1 and 3) 6 mol guanidinium chloride and two pieces of membranes (lane 1 and 2) were incubated with GST-B, 1.0 μg/ml and probed with GST-specific rabbit polyclonal antibodies. The proteins were visualized as described in the legend to Fig. 3. The protein loading was monitored by reaction using anti-keratin antibodies, HRP-conjugated goat antibodies against mouse IgG and a chemiluminescent reagent (lane 3 and 4). After denaturing keratin, the binding ability of the GST-B fusion protein decreased.

us to examine the binding activity to IF depending on the number of linker repeats.

Finally, we also made three fusion proteins using the linker repeat, and these fusion proteins included one, two and three linker repeats. In a slot-blot assay, fusion proteins L1R to L3R, with increasing numbers of linker repeats, bound to keratin with increasing avidity. L3R, which contained three linker repeats, bound to keratin almost as strongly as did the entire linker domain (Fig. 6). These data suggest that the repeated structures of the linker domain also play an important role in the binding of epiplakin to keratin.

DISCUSSION

In this study we showed that the B domain of epiplakin interacts with IF by slot-blot assays. We found that the repeated structure of the B domain plays an important role in the binding to keratin. The linker domain also bound to IF in slot-blot assays, although more weakly than the B domain. The

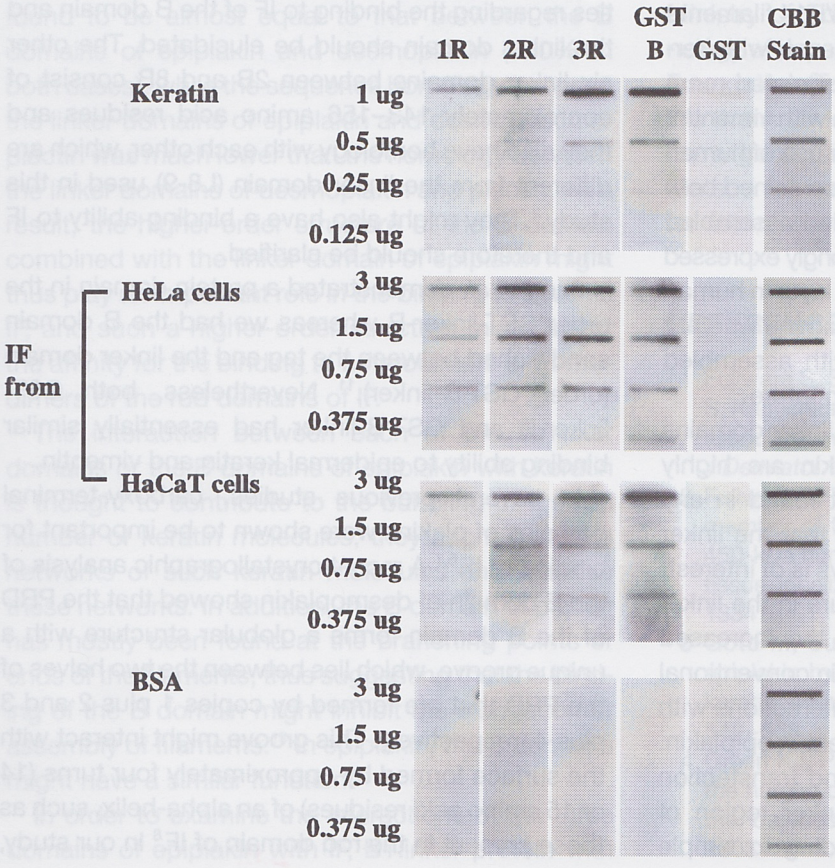

Figure 5. Repeated structures of B domain are important for binding to keratin. For slot-blot assays, keratin, intermediate filament (IF) fractions extracted from HeLa cells and HaCaT cells, and BSA (as a control), were immobilized on a PVDF membrane. Each membrane was incubated with 1.0 μg/ml of GST-fusion proteins that included one, two and three copies of PRD (1R-3R as shown in Fig. 1), GST-B domain and GST (as a control). The proteins were visualized as described in the legend to Fig. 3. The protein loading was monitored by with CBB. 2R and almost 3R in the B domain were necessary for binding to keratin, or to IF extracted from HeLa or HaCaT cells.

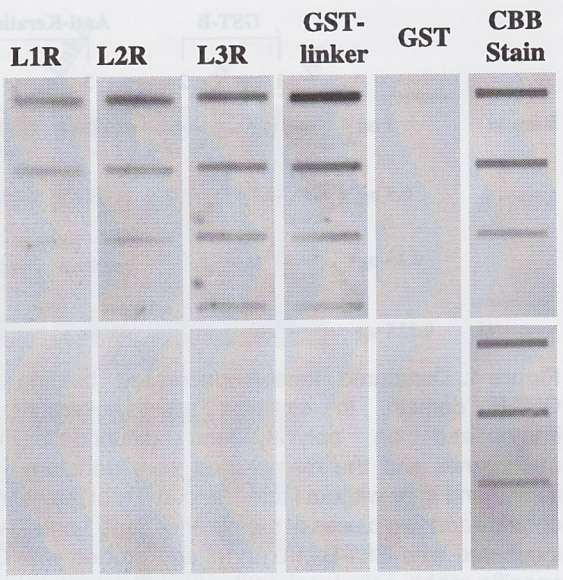

Figure 6. Repeated structures of the linker domain are also important for the binding to keratin. Immobilized keratin or BSA (as a control) on a PVDF membrane was incubated with 3.0 μg/ml of fusion proteins (L1R, L2R, L3R and GST-linker) and GST (as a control), as shown schematically in Fig. 1. The proteins were visualized as described in the legend to Fig. 3. The protein loading was monitored by with CBB. L3R that included three linker repeats bound to keratin almost as strongly as the entire linker domain, while L2R and L1R to keratin bound less strongly in that order.

repeated structure of the linker domain was found to play an important role in the binding to keratin.

Our results seem to be at variance with those of a previous study of Jang *et al.*, which indicated that the B domain is able to interact with vimentin relatively stronger than the assembled K5/K14 filaments and the linker domain is unable to interact with keratin.[11] In our study, the B domain associated more preferably with epidermal keratin than with vimentin. This phenomenon might be due to our use of human epidermal keratin, which presumably contained both K1/K10 and K5/K14 instead of purified assembled K5/K14 filaments. Epiplakin is more strongly expressed in the spinous layers than in the basal layer in human epidermis.[2] As a result, we should examine whether epiplakin interacts more strongly with assembled K1/K10 than with K5/K14 filaments in future.

The repeated structures of the five linker domains in the carboxy terminus of epiplakin are highly unique and such structures are not found in any other plakin family. Our observation that the linker domain bound to IF in slot-blot assays is of interest, particularly that the repeated structure in the linker domain of epiplakin is involved in the increased binding to IF. The linker subdomains in conventional plakins are known to participate in interactions with IF[9] but such sequences are not present in epiplakin. Using two- or three-hybrid assay and transfection assay, Fontao *et al.* showed the linker region of desmoplakin to be involved in binding to simple epithelial keratin K8/K18 and vimentin but not to epidermal keratin K5/K14.[10] Although it is not known whether the linker domain of epiplakin binds to simple epithelial keratin K8/K18 or some other keratin heterodimer (e.g. K6/K16 or K6/K17)[3], the specificities regarding the binding to IF of the B domain and the linker domain should be elucidated. The other six linker domains between 2B and 8B consist of approximately 143–156 amino acid residues and they also have homology with each other, which are different from the linker domain (L8-9) used in this study.[2] They might also have a binding ability to IF and therefore should be clarified.

Jang *et al.* demonstrated a protein domain in the order GST-linker-B whereas we had the B domain sandwiched between the tag and the linker domain (order GST-B-linker).[11] Nevertheless, both GST-linker-B and GST-B-linker had essentially similar binding ability to epidermal keratin and vimentin.

In several previous studies, carboxy-terminal domains of plakins were shown to be important for binding to IF.[6,7] A recent crystallographic analysis of the B domain of desmoplakin showed that the PRD of the B domain forms a globular structure with a unique groove, which lies between the two halves of the PRD that are formed by copies 1 plus 2 and 3 plus 4, respectively. This groove might interact with the surface formed by approximately four turns (14 or 15 amino acid residues) of an alpha-helix, such as those present in the rod domain of IF.[8] In our study,

at least two and closer to three copies of the 4.6 copies in the B domain were necessary to achieve the binding to keratin and these data are consistent with the model described by Choi *et al.*[8] However, Choi *et al.* found that the linker region of desmoplakin did not contribute significantly to the binding affinity but might provide some flexibility that allows the combination of the B and C domains to bind more strongly than to either domain individually. The three repeats of the linker domains of epiplakin are necessary for binding to IF as shown in this study. The linker domains also seem to be globular, similar to the B domain,[2] and the findings of an analogous study using crystallographic analysis on the linker domain might support our finding.

The binding of B+linker domain to polymeric IF was the strongest in the case of keratin, and weaker in the case of vimentin and then desmin, resembling the binding of desmoplakin to these proteins.[17] The B domain with some linker region of plectin associates with vimentin more strongly than with keratin, especially K5/14.[18,19] The sequence homology between the B domains of epiplakin and plectin was found to be almost equal to that between the B domains of epiplakin and desmoplakin (~70% in both cases), while the sequence homology between the linker domains of epiplakin and desmoplakin or plectin was much lower than the homology between the linker domains of desmoplakin and plectin. As a result, the higher-order structure of the B domain combined with the linker domain of epiplakin might thus play an important role in the binding to specific IF, and such a higher-order structure might affect the affinity for the binding to heterodimers or homodimers of the rod domains of IF.

The interaction between each of the B+linker domains or the B domains of epiplakin with keratin is thought to contribute to the bundling of a small number of keratin molecules; they might generate networks of such keratin molecules and stabilize these networks. In addition, the B domain of plectin has mostly been found at the branching points or ends of the filaments, thus suggesting that the binding of the B domain might inhibit the elongation or assembly of filaments.[19] In epiplakin, the B domains might have a similar function.

In order to examine the interactions of the three domains of epiplakin with IF, B+linker protein with tag expressed in 293-EBNA cells was either coimmunoprecipitated with anti-tag antibody and detected by anti-keratin antibody or we performed pull-down assays using fusion proteins and HeLa cell lysates, but failed to identify any interaction with IF or other molecule(s) (data not shown). Even in control experiments using GST, we detected several "pulled-down" proteins, perhaps because of the tendency of IF to aggregate or precipitate. We plan to confirm the binding activity of the repeated structure of the B domain and the linker domain using yeast two-hybrid assay or transfection assay.

In summary, we herein showed that the highly repeated structures of epiplakin in both the B and the linker domains are essential for binding to IF. The crystal structure of the linker domain of epiplakin and quantitative information regarding the binding of these three domains remains to be elucidated.

ACKNOWLEDGMENTS

This work was supported in part by grants (Nos. 1370892, 15591184 and 17591176 to S. F.) from the Ministry of Education, Culture, Science and Technology of Japan and from the Cosmetology Research Foundation (Tokyo).

We thank Dr Hideyuki Saya (Kumamoto University) for a generous gift of HaCaT cells, Dr Noritaka Matsuo for helpful discussion, and Dr Mizuki Goto for submission of this manuscript.

REFERENCES

1 Fujiwara S, Shinkai H, Takayasu S, Owaribe K, Tsukita S, Kageshita T. A case of subepidermal blister disease associated with autoantibody against 450-kDa protein. *J Dermatol* 1992; **19**: 610–613.

2 Fujiwara S, Takeo N, Otani Y, *et al.* Epiplakin, a novel member of the plakin family originally identified as a 450-kDa human epidermal autoantigen: Structure and tissue localization. *J Biol Chem* 2001; **276**: 13340–13347.

3 Goto M, Sumiyoshi H, Sakai T *et al.* Elimination of epiplakin by gene targeting results in acceleration of keratinocyte migration in mice. *Mol Cell Biol* 2006; **26**: 548–558.

4 Green KJ, Parry DAD, Steinert PM, *et al.* Structure of the human desmoplakins. Implications for function in the desmosomal plaque. *J Biol Chem* 1990; **265**: 2603–2612.

5 Wiche G, Becker B, Luber K, *et al.* Cloning and sequencing of rat plectin indicates a 466-kD polypeptide chain with a three-domain structure based on a central alpha-helical coiled coil. *J Cell Biol* 1991; **114**: 83–99.
6 Wiche G, Gromov D, Donovan A, Castanon MJ, Fuchs E. Expression of plectin mutant cDNA in cultured cells indicates a role of COOH-terminal domain in intermediate filament association. *J Cell Biol* 1993; **121**: 607–619.
7 Meng JJ, Bornslaeger EA, Green KJ, Steinert PM, Ip W. Two-hybrid analysis reveals fundamental differences in direct interactions between desmoplakin and cell type-specific intermediate filaments. *J Biol Chem* 1997; **272**: 21495–21503.
8 Choi HJ, Park-Snyder S, Pascoe LT, Green KJ, Weis WI. Structures of two intermediate filament-binding fragments of desmoplakin reveal a unique repeat motif structure. *Nat Struct Biol* 2002; **9**: 612–620.
9 Karashima T, Watt FW. Interaction of periplakin and envoplakin with intermediate filaments. *J Cell Sci* 2002; **115**: 5027–5037.
10 Fontao L, Favre B, Riou S, *et al.* Interaction of the bullous pemphigoid antigen 1 (BP230) and desmoplakin with intermediate filaments is mediated by distinct sequences within their COOH terminus. *Mol Biol Cell* 2003; **14**: 1978–1992.
11 Jang SI, Kalinin A, Takahashi K, Marekov LN, Steinert PM. Characterization of human epiplakin: RNAi-mediated epiplakin depletion leads to the disruption of keratin and vimentin IF networks. *J Cell Sci* 2005; **118**: 781–793.
12 Takeo N, Wang W, Matsuo N, Sumiyoshi H, Yoshioka H, Fujiwara S. Structure and heterogeneity of the human gene for epiplakin (EPPK1). *J Invest Dermatol* 2003; **121**: 1224–1226.
13 Frangioni JV, Neel BG. Solubilization and purification of enzymatically active Glutathione S-Transferase (pGEX) fusion proteins. *Anal Biochem* 1993; **210**: 179–197.
14 Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J Cell Biol* 1988; **106**: 761–771.
15 Izawa I, Nishizawa M, Ohtakara K, Ohtsuka K, Inada H, Inagaki M. Identification of Mrj, a DnaJ/Hsp40 family protein, as a keratin 8/18 filament regulatory protein. *J Biol Chem* 2000; **275**: 34521–34527.
16 Spazierer D, Fuchs P, Proll V, *et al.* Epiplakin gene analysis in mouse reveals a single exon encoding a 725-KDa protein with expression restricted to epithelial tissues. *J Biol Chem* 2003; **278**: 31657–31666.
17 Kouklis PD, Hutton E, Fuchs E. Making a connection: direct binding between keratin intermediate filaments and desmosomal proteins. *J Cell Biol* 1994; **127**: 1049–1060.
18 Nikolic B, Nulty EM, Mir B, Wiche G. Basic amino acid residue cluster within nuclear targeting sequence motif is essential for cytoplasmic plectin-vimentin network junctions. *J Cell Biol* 1996; **134**: 1455–1467.
19 Steinboeck FA, Nikolic B, Coulombe PA, Fuchs E, Traub P, Wiche G. Dose-dependent linkage, assembly inhibition and disassembly of vimentin and cytokeratin 5/14 filaments through plectin's intermediate filament-binding domain. *J Cell Sci* 2000; **113**: 483–491.