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Identification of a novel secretory leukocyte protease inhibitor-binding protein involved in membrane phospholipid movement

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Abstract Previous studies have suggested that human salivary secretory leukocyte protease inhibitor (SLPI) inhibits HIV-1 by binding to a host cell surface protein of unknown identity. Using the yeast two-hybrid assay, we identified a gene sequence encoding a novel SLPI-binding protein (SLPI-BP). The 1.5-kb cDNA encodes a 318-amino acid protein with a predicted transmembrane segment near the C-terminus. Sequence analysis revealed that SLPI-BP is the human scramblase protein that is involved in the movement of membrane phospholipids. Co-expression of SLPI and SLPI-BP followed by an S-protein pulldown assay confirmed the specific interaction between these two proteins. Our data represent the first report for the identity of SLPI-BP.

Key words: Secretory leukocyte protease inhibitor-binding protein; Scramblase; Saliva; Human immunodeficiency virus; Protein–protein interaction

1. Introduction

The secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor found in fluids lining mucosal surfaces. Human salivary SLPI was first identified in parotid saliva as a protein immunologically related to the bronchial mucus leukocyte protease inhibitor [1]. The protein consists of two homologous cysteine-rich domains [2] and exhibits inhibitory activity against neutrophil elastase, chymotrypsin, trypsin and cathepsin G [1,3]. In addition to its anti-protease and anti-inflammatory activities, salivary SLPI is also a potent HIV-1 inhibitor that inhibits HIV-1 infection in macrophages at physiological concentrations [4]. However, SLPI does not appear to bind HIV-1 viral particle, inhibit HIV-1 reverse transcriptase or protease, or transduce an activation signal or downregulate expression of the HIV-1 receptor, CD4 [4]. Rather, its target may be a host cell-associated molecule whose identity is presently not known [4,5].

In this study, we report the identification of a cDNA encoding a SLPI-binding protein (SLPI-BP) by the in vivo yeast two-hybrid analysis. Our results indicate that scramblase, a membrane protein responsible for the dynamic movement of membrane phospholipids (PLs), interacts with SLPI. The potential role of scramblase in HIV pathogenesis is discussed.

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2. Materials and methods

2.1. Materials

The pCI-neo vector was purchased from Promega (Madison, WI, USA). The Matchmaker Two-Hybrid System-2 kit was purchased from Clontech (Palo Alto, CA, USA). The Thermo Sequenase ^{33}P-labeled Terminator Cycle Sequencing Kit was purchased from Amersham (Arlington Heights, IL, USA). The lipofectamine reagent was purchased from Life Technologies (Grand Island, NY, USA). S-protein agarose was purchased from Novagen (Madison, WI, USA).

2.2. Plasmid construction

To generate the GAL4 DNA-binding domain (BD)–SLPI hybrid construct, a recombinant baculovirus shuttle vector containing SLPI cDNA [6] was used as a template in a polymerase chain reaction (PCR) to incorporate an *Nco*I restriction site downstream to the SLPI signal peptide sequence. The 400-bp amplified SLPI cDNA was purified from agarose gel and subcloned into the *Nco*I/*Pst*I sites of the pAS2-1 to obtain pAS2-1/SLPI. To construct the SLPI mammalian expression vector, the DNA fragment encoding for a His-S-tag SLPI fusion protein was isolated from our previously constructed *Escherichia coli* expression vector pET30C(+) and subcloned into the *Xba*I/*Not*I sites of pCI-neo plasmid to obtain the pCI-neo/His-S-SLPI. To construct the SLPI-BP mammalian expression vector, the SLPI-BP cDNA was first subcloned into a previously constructed pCI-neo/T7-P82 plasmid that contains a T7-tag sequence upstream of the multiple cloning sites [7]. The resulting T7-SLPI-BP DNA fragment was then subcloned into the *Xba*I/*Not*I sites of the pCI-neo plasmid to create pCI-neo/T7-SLPI-BP.

2.3. Yeast two-hybrid screening of the peripheral blood leukocyte cDNA library

To identify leukocytic proteins that interact with salivary SLPI, the Matchmaker Two-Hybrid System-2 kit was used. The full-length SLPI cDNA inserted into the GAL4 DNA-BD vector pAS2-1 was used as bait in screening of the phytohemagglutinin-stimulated human leukocyte cDNA library (library size 3×10^6) that was fused to the GAL4 activation domain (AD) vector pGAD10. The GAL4 BD/SLPI hybrid construct (i.e. pAS2-1/SLPI) and the AD/leukocyte library hybrids were co-transformed into yeast strain CG-1945 (Trp^-, Leu^-) for library screening. Transformants were selected for those that contain both types of plasmids with both functional TRP1 and LEU2 gene restored (i.e. Trp^+, Leu^+) and also express interacting hybrid proteins (i.e. His^+). Primary His^+ transformants were then tested for true interacting clones by screening for expression of the second reconstituted reporter gene lacZ in a β-galactosidase assay as described below. Plasmids from putative His^+LacZ^+ positive clones were isolated from the yeast and then individually transformed into *E. coli* HB101 cells for amplification. To eliminate false positive, these plasmids were separately introduced into either the CG-1945 or Y187 yeast strains. The resulting yeast transformants were then tested by either sequential transformation or yeast mating assay with yeast strains containing the GAL4 BD/SLPI. The co-transformant diploid cells were tested for β-galactosidase activity. Only the plasmids that conferred positive expression were analyzed. Clones containing cDNA for possible SLPI-BP of interest were screened by PCR and the amplification products were analyzed in 1% agarose gel to estimate the size.

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Subsequently, plasmids were harvested from the positive clones and re-examined for their ability to bind to SLPI in S-protein pulldown assays. The sequence of the positive cDNA clones was determined by dideoxynucleotides chain termination method using the Thermo Sequenase ^{33}P-labeled Terminator Cycle Sequencing Kit and was analyzed by the BLAST Network Service at NCBI.

2.4. Filter lift LacZ assay

The filter-lift LacZ assay is a qualitative blue/white screening procedure. We performed this assay to screen for reconstitution of the LacZ reporter gene in cotransformants that survive the HIS3 growth selection in a GAL4 two-hybrid library screening. The His^+ colonies of the yeast strain CG-1945 were patched on synthetic dextrose (SD) dropout plates (Trp^-, Leu^- and His^-) supplemented with 5 mM 3-AT. The plates were incubated at 30°C for 1–2 days. A dry sterile Whatman #5 filter was placed over the surface of colonies to be assayed and then transferred to a pool of liquid nitrogen for 10 s followed by thawing at room temperature to permeabilize the cells. The filter was placed on a filter presoaked in 2.5–5 ml of Z buffer/X-gal solution at 30°C and checked periodically for the appearance of blue colonies.

2.5. Yeast mating assay

pAS2-1/SLPI and control plasmids (pAS2-1 and pLAM5′ (a hybrid plasmid expressing a fusion of the DNA-BD and an unrelated protein)) were separately transformed into yeast strain Y187 (MATα), while each candidate AD/library plasmid to be tested was transformed into yeast strain CG-1945 (MATa). The resulting Trp^+ Leu^+ transformants were allowed to mate in 0.5 ml of YPD medium at 30°C with shaking at 250 rpm for 6–18 h. The mating culture was then plated on 100-mm, SD/−Leu/−Trp and SD/−Leu/−Trp/−His/+5 mM 3-AT plates. The plates were incubated at 30°C for 3–5 days to allow diploid cells to form visible colonies. Colonies were scored for growth on the SD/−Leu/−Trp/−His/+3-AT plates.

2.6. Antibody production against SLPI-BP C-terminal peptide

Antiserum was raised in rabbits using a peptide corresponding to a sequence in the C-terminus of SLPI-BP (CESTGSQEQKSGVW) conjugated to keyhole limpet hemocyanin. The specificity of the antibody towards the SLPI-BP was determined by Western blot analysis using T7-SLPI-BP transfected COS cells. As control, parental COS cells or mock transfected cells were used.

2.7. Transient transfection and S-protein pulldown assays

COS cells were transfected with 2 μg of the indicated plasmids (pCI-neo/His-S-SLPI and/or pCI-neo/T7-SLPI-BP) using 6 μl of lipofectamine reagent as previously described [7]. Forty-eight h after transfection, total cell lysates were obtained by extracting the cells with 200 μl lysis buffer A (50 mM HEPES, pH 7.5, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 200 μM sodium orthovanadate, 0.01 M sodium fluoride and 1 mM EGTA) at 4°C for 30 min. The lysates were cleared by centrifugation.

S-Protein pulldown assays were performed by mixing 400 μl of cell lysates with 50 μl of agarose-conjugated S-protein at 4°C for 2 h. After three washes with the lysis buffer A, the pellets were dissolved in 1×sodium dodecyl sulfate (SDS) sample buffer and the supernatants were subjected to Western blot analysis using an anti-C/304–318 antibody.

2.8. Western blot analysis

The expression of protein was detected by the enhanced chemiluminescence (ECL) method as described previously [8]. Briefly, cell lysates were fractionated on a 12% SDS–polyacrylamide gel. After electrotransferring to a PVDF membrane, the non-specific binding sites were blocked by immersing the membrane into a blocking buffer containing 5% dry milk in phosphate-buffered saline and 0.1% Tween 20 (PBS-T) at room temperature for 2 h. Then the membrane was incubated with the primary antibody diluted in the blocking buffer for 1 h. After washing the membrane for 20 min with PBS-T, the horseradish peroxidase-labeled secondary antibody was added and incubated for 1 h at room temperature. After the final wash, the detection reagents were mixed and applied to cover the surface of the membrane. For autoradiography, film was exposed as described by the manufacturer.

3. Results

3.1. Isolation of SLPI-BP in a yeast two-hybrid library screening

To identify leukocytic proteins that interact with salivary SLPI, we used the in vivo yeast two-hybrid system. A construct with GAL4 BD fused with SLPI (i.e. pAS2-1/SLPI) was co-transformed with the human peripheral blood leukocyte cDNA library fused with GAL4 AD into yeast strain CG-1945. Positive clones were selected by auxotrophy for histidine and by expression of β-galactosidase. Approximately 3×10^6 independent clones were screened. Initial screenings of the leukocyte cDNA library had generated 50 putative His^+ clones. Among these primary His^+ clones, the true interacting clones were screened for expression of the second reconstituted reporter gene (*LacZ*) using the β-galactosidase assay. Six clones exhibiting His^+LacZ^+ positive interaction phenotypes were identified. DNA sequencing analysis revealed that five of the His^+LacZ^+ clones (designated His 2, 8, 9, 13 and 14) are of the same cDNA species with different lengths of the untranslated and/or translated regions (Fig. 1). BLAST se-

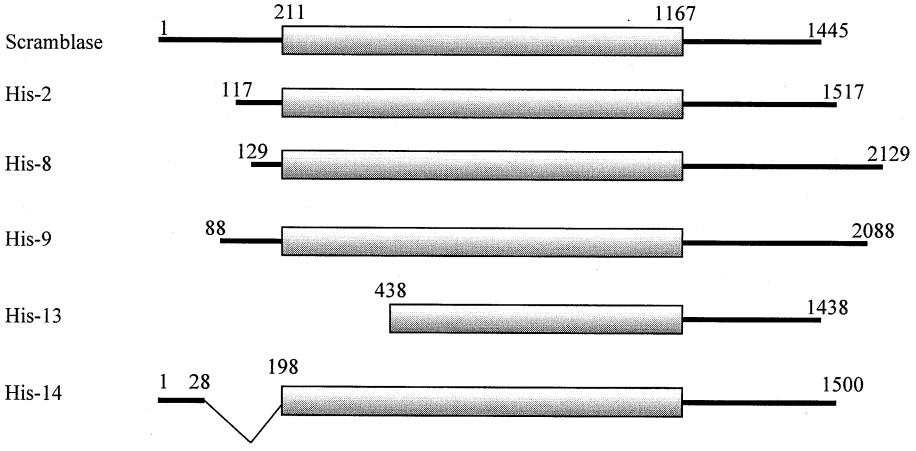

Fig. 1. Alignment of the nucleic acid sequences of the His^+LacZ^+ SLPI-BP clones and scramblase (access number AF008445). Numbers above the sequence refer to the nucleotide numbering of scramblase. The solid bars indicate regions of predicted translated sequence, while the line regions denote the predicted untranslated region.

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GAA TTC GCG GCC CGC GTC GAC GGC AGT CTA GAG GCG CAG AAG AGG AAG CCA TCG CCT
GGC CCC GGC TCT CTG GAC CTT GTC TCG CTC GGG AGC GGA AAC AGC GGC AGC CAG AGA
ACT GTT TTA ATC ATG GAC AAA CAA AAC TCA CAG ATG AAT GCT TCT CAC CCG GAA ACA
AAC TTG CCA GTT GGG TAT CCT CCT CAG TAT CCA CCG ACA GCA TTC CAA GGA CCT CCA
GGA TAT AGT GGC TAC CCT GGG CCC CAG GTC AGC TAC CCA CCC CCA CCA GCC GGC CAT
TCA GGT CCT GGC CCA GCT GGC TTT CCT GTC CCA AAT CAG CCA GTG TAT AAT CAG CCA
GTA TAT AAT CAG CCA GTT GGA GCT GCA GGG GTA CCA TGG ATG CCA GCG CCA CAG CCT
CCA TTA AAC TGT CCA CCT GGA TTA GAA TAT TTA AGT CAG ATA GAT CAG ATA CTG ATT
CAT CAG CAA ATT GAA CTT CTG GAA GTT TTA ACA GGT TTT GAA ACT AAT AAC AAA TAT
GAA ATT AAG AAC AGC TTT GGA CAG AGG GTT TAC TTT GCA GCG GAA GAT ACT GAT TGC
TGT ACC CGA AAT TGC TGT GGG CCA TCT AGA CCT TTT ACC TTG AGG ATT ATT GAT AAT
ATG GGT CAA GAA GTC ATA ACT CTG GAG AGA CCA CTA AGA TGT AGC AGC TGT TGT TGT
CCC TGC TGC CTT CAG GAG ATA GAA ATC CAA GCT CCT CCT GGT GTA CCA ATA GGT TAT
GTT ATT CAG ACT TGG CAC CCA TGT CTA CCA AAG TTT ACA ATT CAA AAT GAG AAA AGA
GAG GAT GTA CTA AAA ATA AGT GGT CCA TGT GTT GTG TGC AGC TGT TGT GGA GAT GTT
GAT TTT GAG ATT AAA TCT CTT GAT GAA CAG TGT GTG GTT GGC AAA ATT TCC AAG CAC
TGG ACT GGA ATT TTG AGA GAG GCA TTT ACA GAC GCT GAT AAC TTT GGA ATC CAG TTC
CCT TTA GAC CTT GAT GTT AAA ATG AAA GCT GTA ATG ATT GGT GCC TGT TTC CTC ATT
GAC TTC ATG TTT TTT GAA AGC ACT GGC AGC CAG GAA CAA AAA TCA GGA GTG TGG TAG
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Fig. 2. DNA sequence of a representative His^+LacZ^+ SLPI-BP clone. The start codon (ATG) and stop codon (TAG) are denoted in bold.

quence similarity searching of the Genbank database revealed that these five clones encoded the human scramblase protein (Fig. 2). With the exception of clone His-13, which encoded an N-terminal truncated protein (deletion of amino acids 1–76), the other four clones encoded the full-length human scramblase with different lengths in the 5′- and/or 3′-untranslated regions (Fig. 1). In addition, all of the five clones showed in-frame fusion with the GAL4 AD (data not shown), suggesting the correct expression of SLPI-BP/scramblase protein in these constructs. With most of the true positive clones encoding scramblase cDNA, these results strongly suggest that scramblase is a major SLPI-BP.

3.2. Verification of positive two-hybrid interactions

The specificity of the interaction between SLPI and SLPI-BP was further verified by the following experiments (Table 1 and Fig. 3). At first, we employed three bait plasmids (pAS2-1 expressing GAL4-DNA-BD, pAS2-1/SLPI expressing GAL4-DNA-BD/SLPI fusion protein, and pLAM5′ expressing GAL4-DNA-BD/human laminin C fusion protein) and four prey plasmids (pGAD10 expressing GAL-AD, pGAD10/library expressing GAL-AD/leukocyte library hybrids, pCL-1 expressing full-length GAL-AD, and pGAD10/SLPI-BP expressing GAL4-AD/SLPI-BP fusion protein) in a set of yeast two-hybrid assays. The SLPI-BP cDNAs were from clones His-2, 8, 9, 13 or 14, respectively. Our results showed that when the yeast strain CG-1945 containing pAS2-1 was transformed with either pGAD10 or pGAD10/library, the transformants did not grow on histidine-free synthetic dextrose (SD/−His) plates (Table 1). Furthermore, yeast harboring a combination of pAS2-1/SLPI and pGAD10 also did not grow on SD/−His plates. In contrast, yeast containing a combination of pAS2-1/SLPI and pGAD10/SLPI-BP grew on SD/−His plates (Table 1).

We then determined the β-galactosidase activity in yeast CG-1945 strains that harbored the same combinations of plasmids as described in Table 1 and Fig. 3. The yeast transformants that contain either (i) pAS2-1/SLPI and pGAD10/SLPI-BP, (ii) pCL-1, (iii) pVA3-1 and pTD1-1 or (iv) pLAM5′ with pCL1, expressed β-galactosidase activity (Fig. 3, lanes 6 and

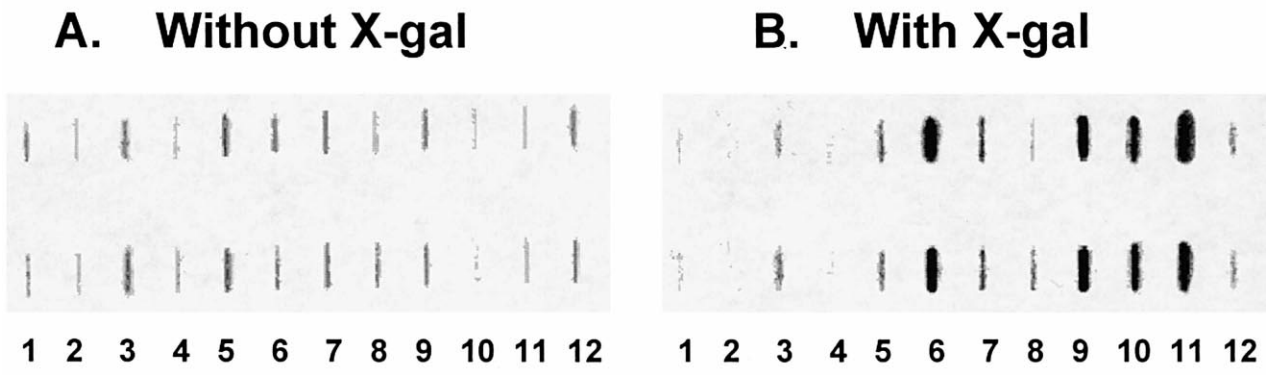

Fig. 3. Association of salivary SLPI and SLPI-BP from leukocyte in the yeast two-hybrid system. The LacZ phenotype expression of the yeast transformants were analyzed in a β-galactosidase assay. Upper and lower rows are duplicates of the same clones. A: Filter paper patched with various yeast colonies to be tested for LacZ phenotype. B: Yeast cells were permeabilized with liquid nitrogen followed by incubation with X-gal on filter at 30°C. The β-galactosidase-producing colonies appeared as dark patches in the figure. The colonies are as follows: 1, pAS2-1/SLPI only; 2, pAS2-1+pGAD10; 3, pAS2-1/SLPI+pGAD10; 4, pGAD10/SLPI-BP; 5, pGAD10 only; 6, pAS2-1/SLPI+pGAD10/SLPI-BP; 7, pGAD10/SLPI-BP; 8, pAS2-1+pGAD10/SLPI-BP; 9, pCL1, a positive control plasmid encoding the full-length, wild-type GAL4 protein; 10, pVA3-1+pTD1-1, which encode for a known interacting protein pair, i.e. a DNA-BD/murine p53 protein and an AD/SV40 large T-antigen protein, respectively; 11, pLAM5′+pCL1. The pLAM5′ is a false-positive detection plasmid which encodes a DNA-BD/human laminin C protein in pAS2-1; 12, pLAM5′+pGAD10/SLPI-BP. The data of pGAD10/SLPI-BP shown here were from clone His-9. Similar results were obtained from clones His-2, 8, 13 and 14 (data not shown).

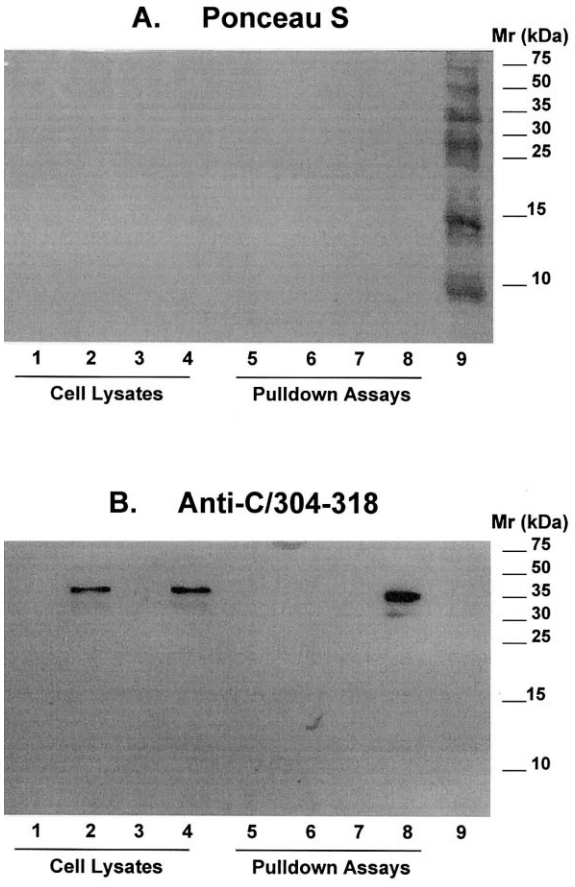

Fig. 4. Interaction of SLPI and SLPI-BP in vivo. The COS cells were transiently transfected with either pCI-neo (lanes 1 and 5), pCI-neo/T7-SLPI-BP+pCI-neo (lanes 2 and 6), pCI-neo/His-S-SLPI+pCI-neo (lanes 3 and 7) or pCI-neo/T7-SLPI-BP+pCI-neo/His-S-SLPI (lanes 4 and 8). After 48 h, the cellular proteins without (lanes 1 to 4) or with (lanes 5 to 8) incubation with agarose-conjugated S-protein were fractionated on a 12% SDS–PAGE and electrotransferred onto a PVDF membrane. A: Blot was stained with Ponceau S to illustrate the equal loading of cell lysates between lanes 1, 2, 3 and 4. B: The same membrane was incubated with an anti-C/304-318 antibody against a C-terminal peptide of SLPI-BP. The sizes of a pre-stained marker are shown in lane 9.

9–11, respectively). Other yeast transformants tested did not express LacZ phenotype. These results indicated that SLPI specifically interacts with SLPI-BP/scramblase.

3.3. In vivo association of SLPI with SLPI-BP

To obtain another indication of the protein–protein interaction suggested by the two-hybrid system, we examined the ability of the protein product of SLPI to 'pull' down SLPI-BP in mammalian cell lysates. At first, we constructed SLPI-BP and SLPI mammalian expression plasmids, pCI-neo/T7-SLPI-BP and pCI-neo/His-S-SLPI, respectively. By design, the SLPI-BP was expressed as a T7-tag fusion protein that could be detected by either the anti-T7 or the anti-SLPI-BP peptide antibody (anti-C/304–318) generated in our laboratory. On the other hand, SLPI was expressed as a His6X and S-tag fusion protein that could be detected by either the anti-His-tag, anti-S-tag, or anti-SLPI antibody. The expression of recombinant T7-SLPI-BP fusion protein was demonstrated in a transient transfection experiment (Fig. 4). After transient transfection of pCI-neo/T7-SLPI-BP into COS cells, a molecular mass of approximately 38-kDa protein was observed for T7-tag SLPI-BP using the anti-C/304–318 antibody (Fig. 4B, lanes 2 and 4). Such protein was absent in the mock transfected cells (Fig. 4B, lane 1), or in cells transfected with pCI-neo/His-S-SLPI (Fig. 4B, lane 3).

To examine the interaction between SLPI-BP and SLPI, COS cells were cotransfected with the following plasmid combinations: (1) pCI-neo; (2) pCI-neo/T7-SLPI-BP+pCI-neo; (3) pCI-neo/His-S-SLPI+pCI-neo or (4) pCI-neo/T7-SLPI-BP+pCI-neo/His-S-SLPI. S-Protein pulldown assays were performed using agarose bead-bound S-protein. Bound proteins were subjected to Western blotting using the anti-C/304–318 antibody (Fig. 4B). We have shown that SLPI-BP was 'pulled' down by S-protein when S-tagged SLPI was co-expressed in the cells (Fig. 4B, lane 8). However, when SLPI-BP was expressed in the absence of S-tagged SLPI, as in the pCI-neo+pCI-neo/T7-SLPI-BP cotransfected cells (Fig. 4B, lane 2), no SLPI-BP protein was detected in the pulldown mixture (Fig. 4B, lane 6). These results indicate that SLPI specifically interacts with scramblase in vivo.

4. Discussion

In this study, we performed a yeast two-hybrid screening using human peripheral blood leukocyte cDNA for candidate proteins that interact with salivary SLPI. Our results suggest that the major SLPI-BP is the human scramblase, a novel 37-kDa integral membrane protein that is involved in the dynamic movement of membrane PLs [9,10]. To the best of our knowledge, the complex between scramblase and a protease inhibitor superfamily has not yet been reported. Thus, our data represent the first report for the identification of scramblase as a SLPI-BP.

The possibility of interaction between SLPI and scramblase seems to be reasonable because five out of six clones identified encoded the same gene product. The interaction was first verified by yeast two-hybrid screening in a yeast mating assay using plasmids rescued separately from the His^+LacZ^+ yeast clones. Subsequent S-protein pulldown experiments provided another line of evidence for such interaction. In this experi-

Table 1
HIS3 reporter gene expression and β-galactosidase activities of yeast strains harboring various combinations of GAL4 DNA-BD and -AD fusion plasmids

No.	Plasmid 1 (DNA-BD)	Plasmid 2 (AD)	His3 phenotype	LacZ phenotype
1	pAS2-1 (DNA-BD/no insert)	pGAD10 (AD only)	no growth	white
2	pAS2-1/SLPI (DNA-BD/SLPI)	pGAD10 (AD only)	no growth	white
3	pAS2-1	pGAD10/library	no growth	white
4	pLAM5′-1 (DNA-BD/control)	pGAD10/library	no growth	white
5	blank	pCL-1 (full-length GAL4)	no growth	blue
6	pAS2-1/SLPI	pGAD10/SLPI-BP (clones #2, 8, 9, 13 and 14)	positive growth	blue

ment, SLPI-BP was 'pulled' down from COS cells co-expressing exogenous S-tag SLPI with an agarose-conjugated S-protein, indicating that the complex of SLPI and SLPI-BP exists in mammalian cells. Although the interacting motifs between these two proteins need to be further identified, DNA sequencing analysis revealed that one of the five interacting clones (clone His-13) encoded an N-terminal truncated scramblase, suggesting that the 76 amino acids of the N-terminus of SLPI-BP is not critical for binding to SLPI.

The precise biological function of the SLPI-scramblase interaction is presently unclear. Numerous studies [4,11–14] have shown that SLPI protects cultured mononuclear cells against HIV infection at an early stage that occurs after viral binding but before viral reverse transcription. HIV-1 gains entry into susceptible cells by means of fusion of viral and cellular membranes. The fusion process is a complex phenomenon that involves an entire range of biochemical and physical interactions. Several studies have implicated a role of membrane PL in viral entry into the host cells. A leucine zipper-like sequence corresponding to the residues 789–815 from the cytoplasmic tail of the viral gp41 protein was shown to bind and perturb PL bilayers of the infected cells [15]. In addition, fusion peptides derived from the HIV-1 gp41 have been shown to associate with PL membranes and inhibited cell–cell fusion [16]. Thus, any molecules that affect PL movement may modulate HIV-1 infection. It is important to notice that since scramblase may regulate the dynamic flip-flop movement of the PL bilayer of cell membrane, we envisage that scramblase may be directly or indirectly involved with the entry of HIV into host cells. In this context, the identity of scramblase as the SLPI-BP appears to fit well with the current model of SLPI's action on the inhibition of HIV-1 infection. However, whether SLPI interacts with scramblase at the cell surface or intra-cellularly is presently unclear. Based on a previous study which suggested that SLPI might attain its anti-HIV activity by binding to a host cell surface molecule [4], we postulate that SLPI may bind specifically to the extracellular domain (C-terminal to the transmembrane domain) of scramblase and that the SLPI–scramblase interaction may stabilize the membrane PL bilayer such that fusion of HIV with host cellular membrane will be blocked. We are currently determining how and at which step such association could affect HIV infection. When these aspects of scramblase action are made clear, we may obtain a new twist in the mechanism of SLPI-induced inhibition of HIV-1 membrane fusion. Such information can ultimately lead to novel therapeutic strategies against HIV and similar viruses.

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