SKAP-HOM, a novel adaptor protein homologous to the FYN-associated protein SKAP55

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Abstract A recombinant GST-Fyn-SH2 domain was used to purify proteins from lysates of pervanadate treated EL4 cells. N-terminal sequencing and molecular cloning of one of the purified polypeptides resulted in the identification of a novel adaptor protein that shares strong structural homology to the recently cloned Fyn-associated adaptor protein SKAP55. This protein was termed SKAP-HOM (SKAP55 homologue). Despite their striking homology, SKAP55 and SKAP-HOM have distinct characteristics. Thus, unlike SKAP55, which is exclusively expressed in T lymphocytes, SKAP-HOM expression is ubiquitous. Furthermore, while SKAP55 is constitutively tyrosine phosphorylated in resting human T cells, SKAP-HOM is expressed as a non-phosphorylated protein in the absence of external stimulus but becomes phosphorylated following T cell activation. In addition, SKAP-HOM does not associate with p59^{fyn} in T cells although it represents a specific substrate for the kinase in COS cells. Finally, we demonstrate that, as previously shown for SKAP55, SKAP-HOM interacts with the recently identified polypeptide SLAP-130.

Key words: T lymphocyte; Signal transduction; Protein tyrosine kinase

1. Introduction

In T lymphocytes, the identification and molecular characterization of intracellular signaling molecules possessing modular binding domains represents an important step towards our understanding of T cell receptor (TCR) mediated activation processes. We have recently cloned a novel adaptor protein, SKAP55, which specifically associates with p59^{fyn} in T lymphocytes [1]. SKAP55 has a particular structural organization and comprises a PH domain, a central region containing several potential tyrosine phosphorylation sites, and a carboxy-terminal SH3 domain. Although the function of SKAP55 in TCR signaling remains to be elucidated, we have recently established that the SH3 domain of SKAP55 mediates a direct interaction with the SLP-76 associated adaptor protein SLAP-130 that is believed to be involved in regulation of IL-2 gene expression [2–4]. This suggests that SKAP55 might participate in a T cell activation pathway that involves Fyn, SLP-76, SLAP-130 and Vav [5–7].

Here we describe the molecular cloning and biochemical analysis of an intracellular phosphoprotein which has an almost identical structural organization to SKAP55. Therefore we termed this protein SKAP-HOM. Despite their strong structural homology, SKAP55 and SKAP-HOM clearly have different characteristics which suggests that the two molecules could fulfil overlapping yet distinct functions during T cell activation.

2. Materials and methods

2.1. Cells and antibodies

Cells were grown in RPMI 1640 supplemented with 10% FCS, 1% penicillin-streptomycin and 2% glutamine (Gibco) at 37°C. Resting human T lymphocytes were prepared as described previously [8].

The anti-phosphotyrosine (4G10, Upstate Biotechnology) and anti-FLAG (M2, Kodak) antibodies were used at 1 μg/ml for immunoblot analysis. Rabbit anti-p59^{fyn} and anti-p56^{lck} antiserum (kindly provided by Dr. A. Veillette, McGill Cancer Center, McGill University, Montreal, Canada) was diluted 1:2000 (v/v) for Western blotting. A polyclonal antiserum directed against SKAP-HOM was raised in rabbits immunized with a synthetic peptide corresponding to amino acids 63–84 coupled to keyhole limpet hemocyanin, while the anti-SKAP55 antibodies are directed against amino acids 49–74 of SKAP55, as previously described [1,9]. The antisera specifically detect SKAP55 or SKAP-HOM and exert no crossreactivity as assessed by immunoprecipitation and Western blot analysis which indicated that SKAP55 is not detected/immunoprecipitated by the anti-SKAP-HOM antibodies and vice versa. Anti-SKAP-HOM and anti-SKAP55 antibodies were used at 1:200 or 1:3000 v/v dilution in immunoprecipitation or Western blotting experiments, respectively. For immunoprecipitation experiments, protein A Sepharose purified anti-phosphotyrosine antibody (PY72, kindly provided by Dr. B. Sefton, Salk Institute, San Diego, CA) was covalently coupled to CNBr-activated Sepharose beads (6 mg/ml packed beads).

2.2. Purification and cDNA cloning of SKAP-HOM

Large scale purification of proteins interacting with the SH2 domain of Fyn and microsequencing were essentially performed as previously described for SKAP55 with the exception that the precipitates were obtained from murine EL4 cells [8,10,11].

Oligonucleotides encompassing the putative ATG and stop codons derived from the 5′ and 3′ human EST clones (numbers AA294994 and U46399, respectively) were synthesized and used in polymerase chain reactions to amplify cDNA reverse-transcribed from peripheral blood lymphocytes total RNA. The oligonucleotides 5′-AACATGCCCAACCCCAGCAGCACC-3′ and 5′-CTCTCAAATATCATACATCTCCATTATG-3′ were used as 5′ and 3′ primers, respectively. The resulting fragment was cloned into a suitable vector and its sequence was determined by automatic sequencing of both strands.

2.3. Northern blot analysis

For Northern blot analysis, blots with poly(A)$^+$ RNA from different human tissues (Clontech) were probed with radiolabeled cDNA of SKAP-HOM under high stringency conditions according to the manufacturer's instruction.

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Abbreviations: EST, expressed sequence tag; GST, glutathione *S*-transferase; SKAP55, src kinase associated phosphoprotein of 55 kDa; SLAP-130, SLP76 associated phosphoprotein of 130 kDa; PH, pleckstrin homology; SH2 and 3, src homology 2 and 3; PTK, protein tyrosine kinase; IEF, isoelectric focusing

The nucleotide sequence of human SKAP-HOM has been submitted to the GenBank/EBI Data Bank with accession number AJ004886.

PII: S0014-5793(98)01040-0

2.4. Immunoprecipitation and Western blotting

Cells were left unstimulated or incubated for the indicated times with culture supernatant of mAb C305 (IgM, a generous gift of Dr. A. Weiss, UCSF, San Francisco, CA), or with 0.1 mM Na vanadate + 1 mM H_2O_2 at 37°C. Immunoprecipitation using anti-SKAP55 or anti-SKAP-HOM antibodies or precipitations using beads coupled to anti-phosphotyrosine mAb or to SH2-domain, electrophoresis and Western blotting were performed as previously reported [10].

2.5. Generation of FLAG-tagged SKAP55 and SKAP-HOM constructs and cell transfection

The pEF-BOS expression vectors containing amino-terminal FLAG-tagged cDNAs of SKAP55 or SKAP-HOM were generated by PCR amplification of their full length coding region with a *Bam*HI site at the 5′ end and an *Xba*I site at the 3′ end. The resulting PCR products were ligated in frame with the FLAG epitope in pEF-BOS expression vector, as described [3].

COS cells were transfected according to the DEAE-dextran method, as reported previously [2]. The constructs used were as follows: wild type Fyn (T) or constitutively active Fyn (F) cloned in pSRα expression vector (kindly provided by Dr. A. da Silva, Dana Farber Cancer Center, Boston, MA), and myc-tagged Lck or ZAP70 inserted into the pcDNA3 vector (a gift from Dr. R. Abraham, Mayo Clinic, Rochester, MN).

Stable transfection was performed as described previously [12]. Stable transfectants were selected in the presence of 0.3 mg/ml hygromycin.

3. Results and discussion

Using the isolated SH2 domain of Fyn expressed as a GST fusion protein we have recently purified a novel Fyn-associated protein, SKAP55, from lysate of pervanadate-treated Jurkat cells [1]. The same experimental procedure was applied to murine EL4 T cells and led to the identification of the murine homologue of SKAP55 and of a second protein spot with slightly higher isoelectric point and apparent molecular mass (not shown). N-terminal sequencing of this protein allowed identification of the first N-terminal amino acids (MPNPSCTSSPGP). This peptide sequence was used to search an EST database with a BLAST algorithm, revealing the existence of a mouse EST clone (number AA268862) that encompassed the coding sequence for the peptide. Computer assisted data base searches allowed identification of the corresponding human EST clone as well as several proximal overlapping clones, from which it was possible to deduce the human cDNA sequence. To verify this sequence, polymerase chain reactions were performed using oligonucleotides corresponding to the putative ATG and stop codons. This resulted in the generation of a ∼1.1 kb fragment. To assess whether this product contains a full open reading frame, 5′ and 3′ rapid amplification of cDNA ends experiments was performed on human leukocyte cDNA. Sequencing of the corresponding products revealed that the 1.1 kb cDNA represents the full coding sequence for the protein (not shown).

Computer assisted analysis of the newly identified protein indicated that it represents a novel protein with a strong structural homology to SKAP55. Indeed, the cDNA encodes a 359 amino acid polypeptide which possesses a PH domain, followed by a central region containing potential tyrosine phosphorylation sites, as well as a carboxy-terminal SH3 domain (Fig. 1). Among the putative tyrosine phosphorylation sites, one fulfils the criteria for being a binding site for SH2 domains of src-PTKs (Y^{251}EEL). Notably, a second tyrosine might represent a binding site for the SH2 domain of Grb2 (Y^{298}AN). In contrast to SKAP55, the novel polypeptide contains an amino-terminal region which would be predicted to form a coiled-coil structure [13]. These domains are thought to mediate non-covalent protein-protein interactions by homo- or hetero-oligomeric associations via charged residues. Because of the strong structural homology to SKAP55 we termed the novel protein SKAP-HOM (SKAP55 homologue).

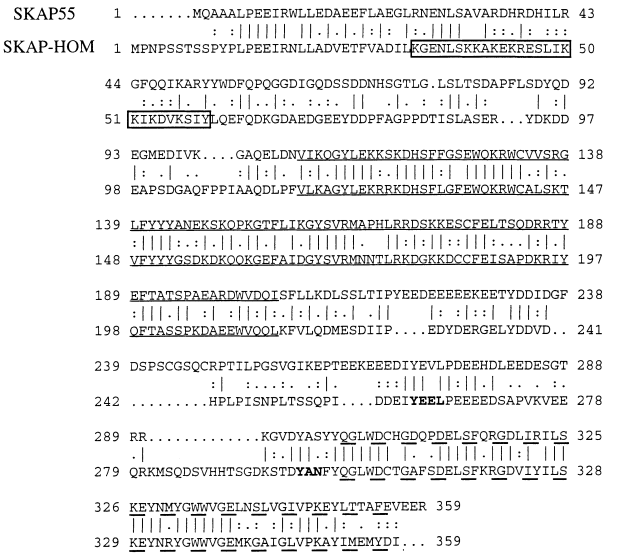

Fig. 1. Comparison of human SKAP55 and SKAP-HOM amino acid sequences. The PH domains are underlined. Dashed lines delimit the SH3 domains. The coiled-coil region of SKAP-HOM is boxed and the putative binding sites for src-kinases (YEEL) and Grb2 (YAN) SH2 domains are in bold.

The Western blot analysis of simultaneously immunoprecipitated SKAP55 and SKAP-HOM shown in Fig. 2 demonstrates that, despite almost identical calculated molecular weights and isoelectric points, SKAP55 and SKAP-HOM can be distinguished from each other by their differential migration in two-dimensional IEF/SDS-PAGE. Thus, SKAP-HOM possesses a slightly more basic isoelectric point and migrates more slowly than SKAP55 (Fig. 2C). Besides SKAP-HOM an additional protein spot migrating at 120 kDa is detected by the polyclonal SKAP-HOM antiserum (Fig. 2B). This protein could represent a 120 kDa polypeptide that crossreacts with the SKAP-HOM antiserum. Alternatively it could represent a dimeric form of SKAP-HOM which is generated via the coiled-coil domain [13].

We previously reported that SKAP55 is exclusively expressed in T lymphocytes [1]. To analyze the expression of SKAP-HOM, Northern blot analysis was performed. A 4.2 kb SKAP-HOM transcript of variable intensity is detected in all tissues that were investigated (Fig. 3A). Two additional transcripts of 1.3 and 2.2 kb are visible in testis which could indicate the existence of alternatively spliced forms of SKAP-HOM or of a highly related gene in this organ. In this regard, it is noteworthy that we also observed a 2.2 kb transcript in testis using SKAP55 cDNA for hybridization [1].

To analyze SKAP-HOM protein expression within the hematopoietic system, various cell lines of hematopoietic origin were stimulated with vanadate, lysed and subjected to precipitation using a GST-Fyn-SH2 fusion protein. The anti-SKAP-HOM Western blot analysis of these precipitates is shown in Fig. 3B and demonstrates that SKAP-HOM protein is detectable in unseparated human T lymphocytes and thymocytes,

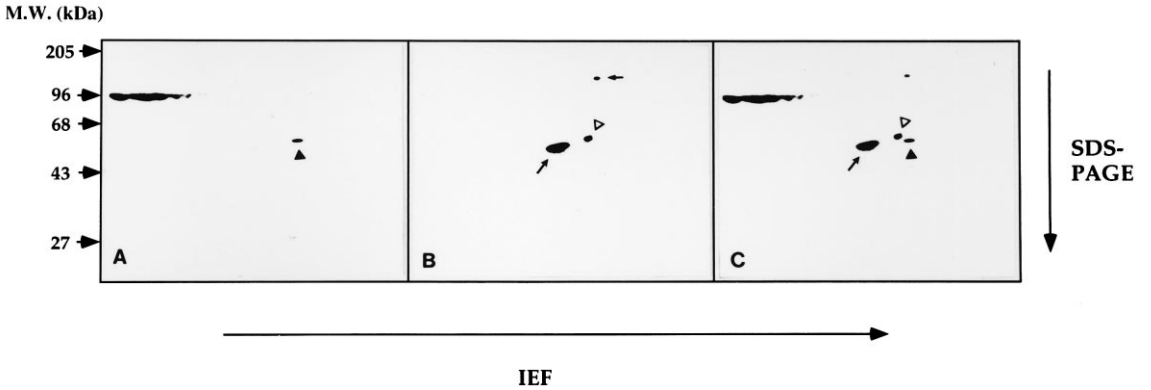

Fig. 2. Two-dimensional IEF/SDS-PAGE analysis of SKAP55 and SKAP-HOM. Immunoprecipitation was performed on lysates of resting human T lymphocytes using a mixture of anti-SKAP55 and anti-SKAP-HOM antisera. Proteins were detected by two-dimensional Western blotting with (A) anti-SKAP55 or (B) anti-SKAP-HOM antibodies. The panel shown in (C) represents an overlay of those presented in (A) and (B). Positions of SKAP55 (black arrowhead) and of SKAP-HOM (open arrowhead) are indicated. The small arrow points at the 120 kDa protein spot which is detected by the anti-SKAP-HOM antiserum. The lower arrow depicts the position of the heavy chain of the antibodies used for precipitation which was determined by probing the blot with the secondary anti-rabbit antibody alone.

the T cell line H9, the murine T cell lines EL-4 and DC28.10, as well as in the recently described $CD8^+$ IL2-dependent cutaneous T cell lymphoma PBLCPCS [14]. In addition, SKAP-HOM is expressed in the B cell lines REH and SKW6.9, in the myeloid cell lines U937 and HL60 and the erythroleukemic cell line K562. Surprisingly, the protein is undetectable in two Jurkat cell clones (Kor.1 and Kab.14), the T cell lines CEM, MOLT4 and HPB-ALL and the Epstein-Barr virus trans-

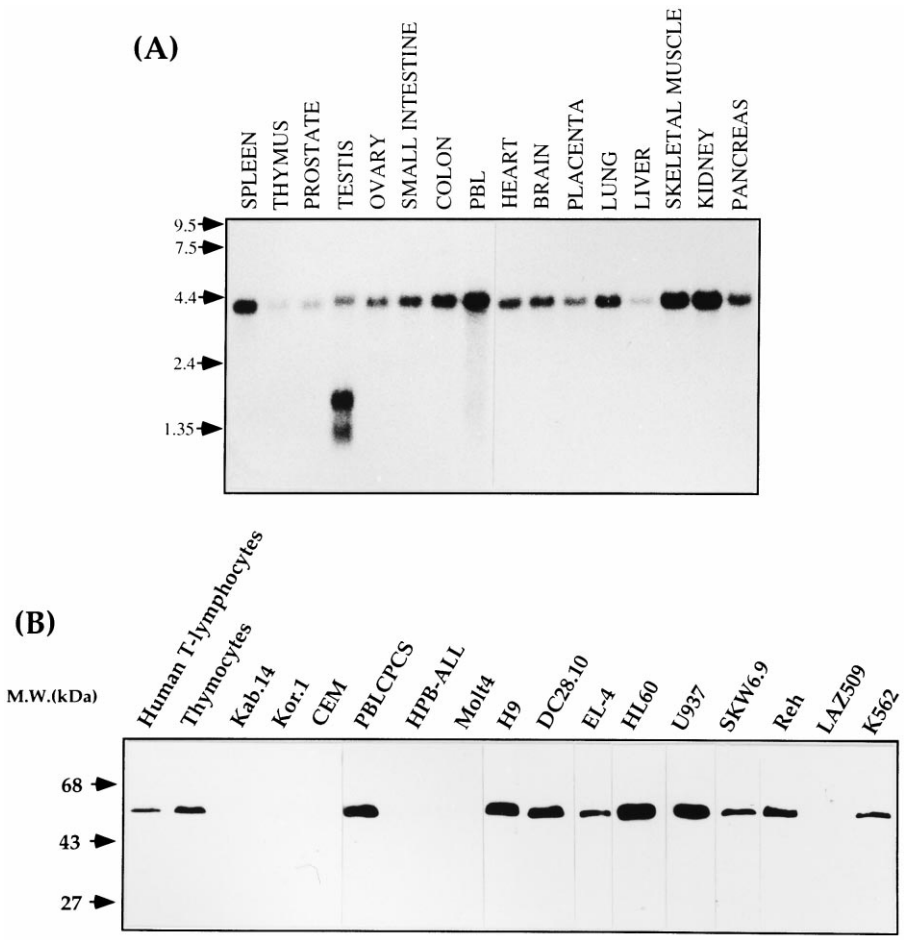

Fig. 3. Tissue and cellular distribution of SKAP-HOM. A: Northern blots containing mRNA from multiple human tissues were probed with a radiolabeled SKAP-HOM cDNA probe and revealed by autoradiography. B: Cell lines of various hematopoietic origins were stimulated with pervanadate, lysed and subjected to precipitation using a GST-Fyn-SH2 fusion protein. Proteins were separated by SDS-PAGE and SKAP-HOM was detected using specific anti-SKAP-HOM antibodies.

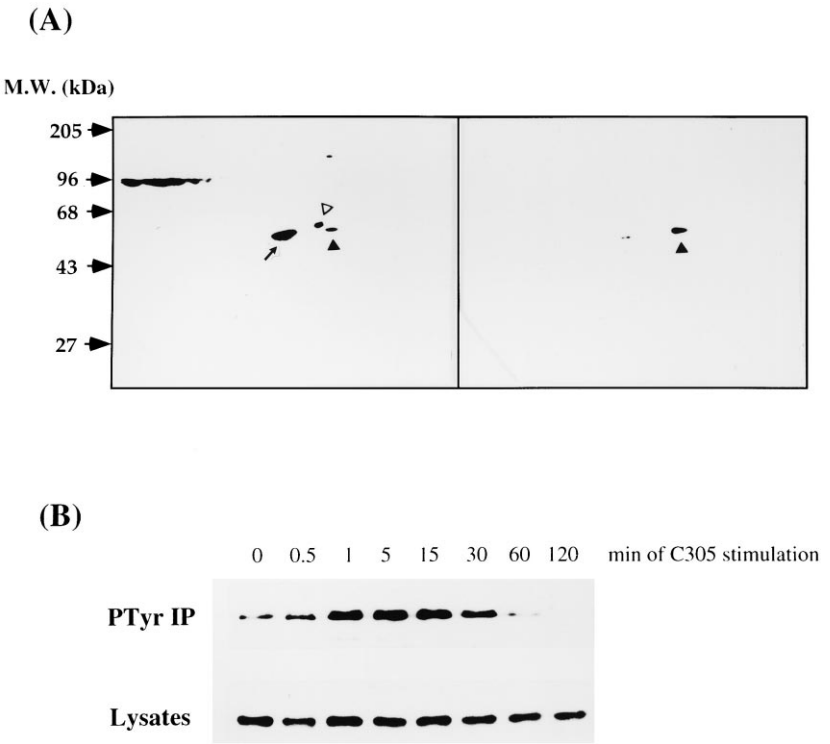

Fig. 4. Tyrosine phosphorylation of SKAP-HOM upon T-cell activation. A: Right panel: The blot shown in Fig. 2 was stripped and reprobed with an anti-phosphotyrosine antibody (4G10). The left panel is identical to the one shown in Fig. 2C. Black and open arrowheads indicate the positions of SKAP55 and SKAP-HOM, respectively. The arrow shows the heavy chain of the precipitating antibodies. B: Jurkat cells were stably transfected with FLAG-tagged SKAP-HOM. Cells were stimulated with C305 for the indicated times, lysed and subjected to anti-phosphotyrosine immunoprecipitation. Immunoprecipitated proteins were resolved on SDS-PAGE and blotted with anti-SKAP-HOM antiserum (upper panel). Lysates corresponding to each time point were analyzed by anti-FLAG immunoblotting (lower panel).

formed B cell line LAZ509. Note that a lack of SKAP-HOM expression correlated with the absence of mRNA (data not shown). Thus, although SKAP-HOM expression does not appear to be restricted to a particular lineage of hematopoietic cells, it is selectively expressed in different clonal populations originating from the same lineage. This could indicate that its expression defines functionally distinct subpopulations within the hematopoietic system.

Fig. 4 demonstrates that SKAP55 and SKAP-HOM also differ in their tyrosine phosphorylation status in resting human T cells. Reprobing of the blot shown in Fig. 2 with an anti-phosphotyrosine antibody allowed detection of a single tyrosine phosphorylated spot exactly co-migrating with SKAP55 (Fig. 4A). This confirms our previous observation that SKAP55 is constitutively phosphorylated on tyrosine residue(s) [1]. In marked contrast, SKAP-HOM is not phosphorylated on tyrosine residue(s) under the same experimental conditions.

To assess whether SKAP-HOM represents a substrate for T cell PTKs we established a Jurkat variant stably expressing a FLAG-tagged SKAP-HOM protein. As shown in Fig. 4B, low amounts of SKAP-HOM are detectable in the anti-phosphotyrosine immunoprecipitates prepared from non-stimulated cells, probably resulting from constitutive activation of PTKs in the transformed cell line. However, a strong increase in SKAP-HOM tyrosine phosphorylation is already detectable following 30 s of stimulation, reaching maximum levels after 5 min of activation (five-fold increase), and then slowly declining. Thus, SKAP-HOM represents a substrate for TCR-activated PTKs in Jurkat T cells.

We next investigated which tyrosine kinases are capable of phosphorylating SKAP55 and SKAP-HOM in vivo. To this end, COS cells were transfected with FLAG-tagged SKAP55 or SKAP-HOM together with constructs encoding Lck, Fyn, and/or ZAP70. Subsequently, anti-phosphotyrosine immunoprecipitates were prepared and analyzed by means of anti-SKAP55 and anti-SKAP-HOM Western blotting. The results depicted in the upper panel of Fig. 5 indicate that both SKAP55 and SKAP-HOM are selectively phosphorylated by wild type Fyn or a constitutively active mutant of the kinase whereas they are not phosphorylated by either Lck or ZAP70 (either alone or in combination). These results indicate that SKAP55 and SKAP-HOM represent specific substrates for Fyn.

SKAP55 constitutively associates with $p59^{fyn}$ in resting human T lymphocytes [1]. To investigate whether this is also true for SKAP-HOM, SKAP55 and SKAP-HOM immunoprecipitates were prepared from lysates of human T lymphocytes and analyzed by Western blotting with Fyn antiserum. As reported previously, Fyn associates with SKAP55 in non-stimulated T lymphocytes (Fig. 6a and [1]). In marked contrast, even under conditions where SKAP-HOM becomes tyrosine phosphorylated (e.g. after pervanadate treatment of the cells, Fig. 6d) no interaction between SKAP-HOM and Fyn is detectable (Fig. 6c). Identical results were obtained when Fyn immunoprecipitates were analyzed by anti-SKAP55 or anti-SKAP-HOM Western blotting (data not shown). This rules out the possibility that the association between Fyn and SKAP-HOM is disrupted by the antibodies used for precipitation. Collectively, the data shown in Figs. 5 and 6 indicate

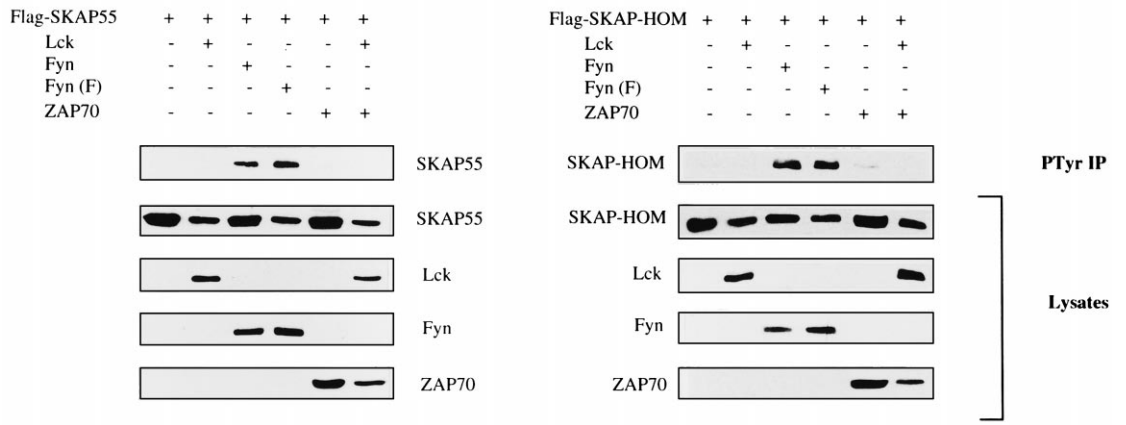

Fig. 5. Tyrosine phosphorylation of SKAP55 and SKAP-HOM by p59^{fyn}. FLAG-tagged SKAP55 or SKAP-HOM in pEF-Bos vector were transfected in COS cells either alone or in combination with Lck, Fyn or ZAP70 constructs as indicated. Fyn (F) is a constitutively active mutant of Fyn. 36 h after transfection, cells were harvested and lysed in NP40 lysis buffer. Aliquots from the lysates were analyzed for expression of SKAP55, SKAP-HOM, Fyn, Lck and ZAP70, as indicated. The remaining lysates were subjected to anti-phosphotyrosine immunoprecipitation (PTyr IP) and the presence of SKAP55 (left panel) or SKAP-HOM (right panel) was assessed by Western blotting using the corresponding specific antibodies.

that, unlike SKAP55, SKAP-HOM likely represents a substrate, but not a ligand, for Fyn. The tight association between SKAP55 and Fyn could account for the constitutive tyrosine phosphorylation of the protein in resting T cells.

We recently established that, in T lymphocytes, SKAP55 directly interacts with the adaptor molecule SLAP-130 via its SH3 domain [4]. Since SKAP55 and SKAP-HOM exert a high degree of homology in their SH3 domain, this raised the possibility that SKAP-HOM also associates with SLAP-130. To assess this hypothesis, anti-SKAP-HOM and anti-SKAP55 immunoprecipitates were prepared from Jurkat cells (which only express SKAP55) or from the H9 T cell line (expressing both protein homologues). As previously demonstrated [3], SKAP55 co-precipitates with SLAP-130 in Jurkat cells (Fig. 6E, lane 1). Perhaps more importantly, even when SKAP55 and SKAP-HOM are concomitantly expressed in the same cell, SKAP-HOM associates with SLAP-130 (Fig. 6E, lanes 3 and 4). A similar association between SKAP-HOM and SLAP-130 is detectable when both proteins are co-expressed in COS cells (not shown). This suggests that the interaction between SKAP-HOM and SLAP-130 is direct and does not involve a third protein.

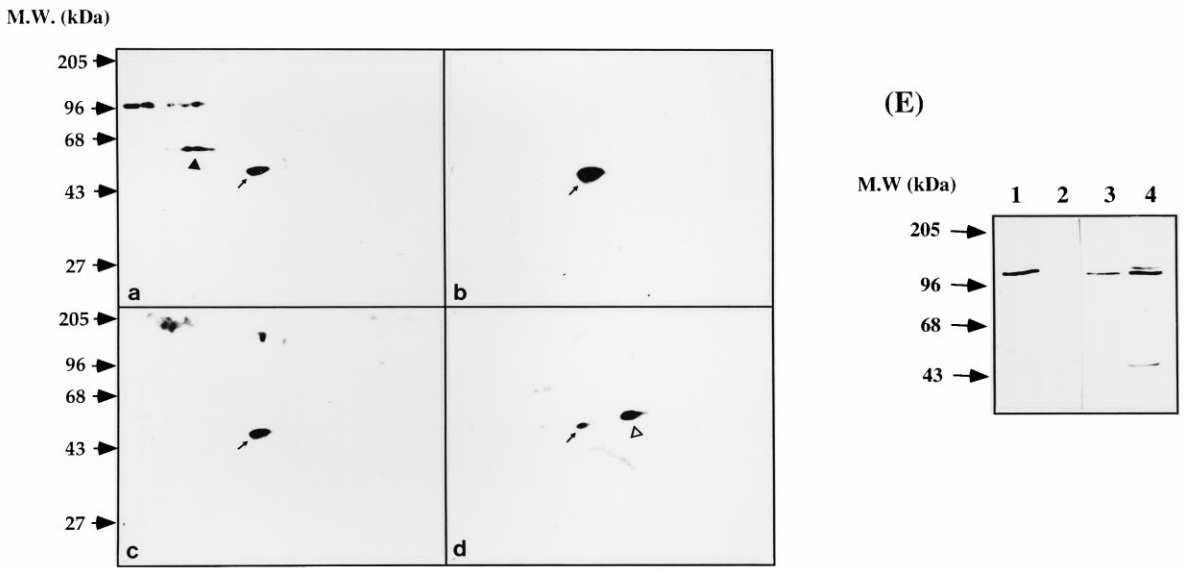

Fig. 6. Analysis of SKAP55 and SKAP-HOM interaction with p59^{fyn} and SLAP-130. Resting T cells were left unstimulated (a and b) or stimulated with pervanadate for 2 min at room temperature (c and d). Lysates were immunoprecipitated with anti-SKAP55 (a) or anti-SKAP-HOM (b–d) antibodies. Samples were resolved on two-dimensional gels and revealed by Western blot using anti-p59^{fyn} (a–c) or anti-phosphotyrosine (d) antibodies. The blot shown in (d) was obtained after stripping the one presented in (c). The black and open arrowheads indicate the positions of Fyn and SKAP-HOM, respectively. The arrow points towards the heavy chain of the immunoglobulins. E: Jurkat (lanes 1 and 2) or H9 (lanes 2 and 3) cells were lysed and subjected to anti-SKAP55 (lanes 1 and 3) or to anti-SKAP-HOM (lanes 2 and 4) immunoprecipitation. Immunoprecipitates were separated on SDS-PAGE and proteins were detected using anti-SLAP-130 antiserum. Note that the upper band detected by the anti-SLAP-130 antibodies in the SKAP-HOM precipitate obtained from H9 cells, and which most likely corresponds to a hyperphosphorylated form of SLAP-130, is also visible in the SKAP55 immunoprecipitates after overexposure of the blot.

In summary, we have identified and cloned a novel adaptor protein, SKAP-HOM, whose structural organization is highly related to SKAP55. While both proteins are potentially capable of interacting with the same intracellular signaling molecules, their specific biochemical characteristics suggest that they may be involved in different pathways of T cell activation. However, our observation that both proteins represent substrates for Fyn and are capable of interacting with SLAP-130 in T cell lines raises the intriguing possibility that in T cells which concomitantly express SKAP55 and SKAP-HOM the two molecules compete with each other for binding to SLAP-130 thereby modulating SLAP-130 function. Since the functions of SKAP55 and SKAP-HOM have not yet been defined and the role of SLAP-130 in TCR mediated signalling events is still a matter of discussion [2,3], additional experiments will be required to establish the precise roles of the SKAP-55/SLAP-130 and SKAP-HOM/SLAP-130 complexes during T cell activation. Nevertheless, given the ubiquitous expression of SKAP-HOM, it seems reasonable to propose that this novel adaptor protein is not only involved in TCR mediated signalling events but also plays a regulatory function outside the hematopoietic system.

3.1. Note added in proof

While this article was under review Liu et al. reported the identification and molecular cloning of a polypeptide termed SKAP55-R which is essentially identical to SKAP-HOM [Liu, J., Kang, H., Raab, M., Da Silva, A., Kraeft, S.-K. and Rudd, C.E. (1998) Proc. Natl. Acad. Sci. USA 95, 8779–8784].

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References

[1] Marie-Cardine, A., Bruyns, E., Verhagen, A.M., Eckerskorn, C., Kirchgessner, H., Meuer, S.C. and Schraven, B. (1997) J. Biol. Chem. 272, 16077–16080.
[2] da Silva, A.J., Li, Z., de Vera, C., Canto, E., Findell, P. and Rudd, C.E. (1997) Proc. Natl. Acad. Sci. USA 94, 7493–7498.
[3] Musci, M.A., Hendricks-Taylor, L.R., Motto, D.G., Paskind, M., Kamens, J., Turck, C.W. and Koretzky, G.A. (1997) J. Biol. Chem. 272, 11674–11677.
[4] Marie-Cardine, A., Hendricks-Taylor, L.R., Boerth, N.J., Zhao, H., Schraven, B. and Koretzky, G.A. (1998) J. Biol. Chem. (in press).
[5] Wu, J., Motto, D.G., Koretzky, G.A. and Weiss, A. (1996) Immunity 4, 593–602.
[6] Tuosto, L., Michel, F. and Acuto, O. (1996) J. Exp. Med. 184, 1161–1166.
[7] Raab, M., da Silva, A.J., Findell, P.R. and Rudd, C.E. (1997) Immunity 6, 155–164.
[8] Schraven, B., Ratnofsky, S., Gaumont, Y., Lindegger, H., Kirchgessner, H., Bruyns, E., Moebius, U. and Meuer, S.C. (1994) J. Exp. Med. 180, 897–906.
[9] Schraven, B. et al. (1994) J. Biol. Chem. 269, 29102–29111.
[10] Marie-Cardine, A., Kirchgessner, H., Eckerskorn, C., Meuer, S.C. and Schraven, B. (1995) Eur. J. Immunol. 25, 3290–3297.
[11] Eckerskorn, C., Mewes, W., Goretski, H. and Lottspeich, F. (1988) Eur. J. Biochem. 176, 509–519.
[12] Bruyns, E., Kirchgessner, H., Meuer, S. and Schraven, B. (1998) Int. Immunol. 10, 185–194.
[13] Lupas, A. (1996) Trends Biochem. Sci. 21, 375–382.
[14] Bagot, M., Echchakir, H., Mami-Chouaib, F., Charue, D., Bernheim, A., Boumsell, L. and Bensussan, A. (1998) Blood 91, 4331–4341.