Vav, a GDP/GTP nucleotide exchange factor, interacts with GDIs, proteins that inhibit GDP/GTP dissociation

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Abstract Vav functions as a specific GDP/GTP nucleotide exchange factor which is regulated by tyrosine phosphorylation in the hematopoietic system. Loss of the amino-terminus sequences of Vav was sufficient to control its transforming potential and its function in T cells. We report here the identification of the hematopoietic GDP dissociation inhibitor protein, Ly-GDI, as a protein that interacts with the amino-terminus of Vav. Further analysis confirmed that Vav and Ly-GDI interact both in in vitro and in in vivo assays. This association is maximal only when the amino region of Vav is intact and requires an intact carboxy-terminus of Ly-GDI. The interaction between Vav and Ly-GDI is not dependent on the tyrosine phosphorylation status of Vav. In addition, Rho-GDI, the highly homologous protein to Ly-GDI, associates with Vav as well. The contribution of the interaction between Vav and GDIs, proteins that are involved in the GDP/GTP exchange processes, to the biological function of Vav is further discussed.

Key words: *vav*; Ly-GDI; GDP/GTP exchange; Rho-GDI

1. Introduction

Although the *vav* proto-oncogene (Vav) was initially identified as an oncogene, accumulating results from the past several years have placed the molecule as an important signal transducer protein, functioning at a pivotal role in hematopoietic cell activation [1–4]. Thus, Vav functions as a specific GDP/GTP nucleotide exchange factor which is regulated by tyrosine phosphorylation in hematopoietic cells, where it is exclusively expressed. [1,2,5–8]. These signaling cascades evoke numerous responses such as capping of the T cell receptor (TCR) following its activation, receptor recruitment of the actin cytoskeleton to the CD3 ζ chain of the TCR, interleukin-2 production and proliferation, cell cycle progression, activity of nuclear factor of activated T cells (NF-AT), phosphorylation of SLP-76 and Ca^{2+} mobilization [3,4]. Thus, in T cells, Vav integrates signals from lymphocyte antigen receptors and costimulatory receptors to control development, differentiation and cell cycle.

Vav encodes a unique protein (95 kDa) that contains a collection of several modular motifs characteristic of tyrosine signal transducer proteins, such as a *dbl* homology domain (DH) that activates GTP-bound proteins of the Rho-like family of proteins [9–11]; a pleckstrin homology domain [12]; a Src homology 2 (SH2) and two Src homology 3 (SH3) domains [13,14]. Additional regions that can be found in Vav are a proline rich motif that might allow it to bind to SH3-containing proteins [1] and an acidic rich region [1]. The leucine rich amino-terminus of Vav is similar to the 'calponin homology' (CH) region (residues 3–115) which functions as an actin-binding protein domain in other proteins [15].

The mechanism by which *vav* transforms fibroblasts is still poorly understood [1,16,17]. Nucleotide sequence analysis of the *vav* oncogene revealed that it was activated in vitro by replacement of 67 residues of its amino-terminus with sequences of pSV2neo, co-transfected as a selectable marker [1]. Vav produces minimal transformation of NIH3T3 murine fibroblasts only when the protein is grossly overexpressed [16,17]. By contrast, removal of its amino-terminus sequences (65 residues), mimicking its original mode of activation, is sufficient to induce *vav* transformation [16,17]. This sequence controls not only *vav* transformation but also its function in T cells. Thus, unlike Vav, the truncated oncogenic protein does not cause any changes in NF-AT transcription [18]. Truncation of the amino-terminus of another member of the Vav family of signaling proteins, *vav*2, also renders it a transforming gene [19]. Hence, Vav probably interacts through its amino-terminus with a regulatory protein which controls both its activity as an oncogene and its function in the proximal TCR signaling pathways.

Indeed, protein–protein interactions were shown to play an important role in the regulation of Vav activity. Thus, mutations of the SH2 and SH3 domains of *vav* were found to impair the transforming activity of the protein and its ability to associate with several proteins [20,21]. Two proteins were reported to associate with the amino-terminus of Vav: Socs1, a downstream component of the Kit receptor tyrosine kinase signaling pathway [22], and ENX-1, a putative transcriptional regulator of homeobox gene expression [23]. However, the contribution of these proteins to the function of Vav is still not known [22,23].

Our attempts to isolate additional proteins that associate with the amino-terminus of Vav by the use of the yeast two hybrid system [24] led us to the isolation of a Vav-binding protein, the GDP dissociation inhibitor protein, Ly-GDI [25,26]. Our results show that an intact amino-terminus of Vav is needed to allow interaction between these two proteins and may explain why an intact amino-terminus of Vav is critical for its activity in oncogenic, mitogenic and signaling events.

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Abbreviations: TCR, T cell receptor; NF-AT, nuclear factor of activated T cells

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2. Materials and methods

2.1. Plasmids

The fragments used for generation of the plasmids (see below) used in this study were obtained by PCR amplification with primers that carry restriction sites for subcloning purposes (underlined). The PCR products were sequenced prior to their use for subcloning.

2.1.1. Yeast two hybrid plasmids. VavY2H$^{1-178}$ carries residues 1–178 of Vav and was created by humN1-5′-CCGGGGAATTCGCCCTGGGCAGGGTAGCC and humN2-5′-GGCCCCGGATCCGCGCATCAGGTCCTCATA. The PCR product was subcloned between the *Eco*RI and *Bam*HI sites of pBTM116. Additional plasmids that were used are pGAD1318 as well as pGAD1318 that carries Lamin (pLex-Lamin).

2.1.2. pGEX-2TK (GST) expression plasmids. (1) GST-Ly carries the entire coding region of Ly-GDI and was prepared by LyGDIF-5′-CCGGGGGGATCCATGACTGAAAAAGCCCCA and LyGDIR-5′-GGCCCCGAATTCATGCATTCATTCTGTCCA. (2) GST-LyN carries the amino-terminus of Ly-GDI (residues 1–59) and was generated by Ly-GDIF and Ly60R-5′-CCGGGGAATTCAGGACCATCTCCCAGCAG. (3) GST-LyC carries the carboxy Ly-GDI (residues 60–201) and was prepared by Ly61F-5′-CCCGGATCCCTGCTGGGAGATGGTCCT and Ly-GDIR. (4) GSTVav$^{1-178}$ carries residues 1–178 of Vav and was amplified with: GST178F-5′-CCGGGGGGATCCGCCCTGGGCAGGGTAGCC and GST178R-5′-GGCCCCGAATTCGCGCATGAGGTCCTCATA. (5) GSTRho-GDI was created by GSTRho-GDIF-5′-CCGGGGGGATCCAGCATGGCTGAGCAGGAG and GSTRho-GDIR-5′-GGCCCCGAATTCTCAGTCCTTCCAGTCCTT. The PCR products were digested with *Bam*HI and *Eco*RI restriction enzymes (underlined above) and subcloned into the glutathione-*S*-transferase (GST) fusion protein vector, pGEX-2TK.

2.1.3. Mammalian expression vectors. Vav and Ly-GDI were subcloned at the *Bam*HI and *Eco*RI sites of the mammalian expression vector driven by the CMV promoter, pCDNA3, thus creating pcDNA3-Vav and pcDNA3-Ly, respectively.

2.2. Cell culture

Cell lines used in this study are: NIH3T3 murine fibroblasts, NIH3T3 cells that express the *vav* oncogene (NIH/vav) [1], NIH3T3 cells that express Vav (NIH/proto-vav) [16]. These cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum. Jurkat T cells were grown in RPMI medium containing 10% fetal calf serum (FCS) and 293T cells were grown in DMEM containing 10% FCS.

2.3. Gene transfer assays

1×10^6 293T cells were transiently transfected with 10 μg DNA of various plasmids (see Section 3) by using the calcium phosphate method. The efficiency of transfection was confirmed by the fluorescence of the green fluorescence plasmid included in the transfection. Cells were washed 12 h following transfection and were harvested 48 h past transfection.

2.4. Immunoprecipitation and immunoblotting

Cells lysis, immunoprecipitation and immunoblotting were performed as previously described [15,16,20,21,27].

2.5. Immobilization of bacterial fusion proteins on glutathione Sepharose beads

Fusion proteins were purified from transformed *Escherichia coli* bacteria and bound to glutathione Sepharose beads (Pharmacia) as previously described [20,21,27].

2.6. Activation of Jurkat T cells

Jurkat T cells at 2×10^7 cells/100 μl were activated with anti-CD3ε monoclonal antibody (Ab) OKT3 (1:100; American Type Tissue Culture Collection) for 1 min at 37°C.

2.7. Abs

Anti-Vav Abs were raised in rabbits against a specific peptide of *vav*, residues 528–541 [16]; anti-phosphotyrosine (Ptyr), 4G10 (UBI); anti-CD3 (American Type Tissue Culture Collection); monoclonal anti-Vav used in Western blots (UBI); and anti-Ly-GDI (UBI).

2.8. Yeast two hybrid system

The *Saccharomyces cerevisiae* L40 cells (*MATa trp1 leu2 his3 LYS::lexA-HIS3 URA3::lexA-lacZ*) were grown in YPD medium (1% yeast extract, 2% polypeptone, 2% glucose) at 30°C. L40 cells were co-transformed sequentially by the lithium acetate method with VavY2H$^{1-178}$ and with a cDNA library from Jurkat cells fused to the GAL4 activation domain in pGAD1318 (kindly given to us by J. Camonis). Double transformants were plated on yeast medium lacking Trp, Leu and His (−LWH) and containing 50 mM 3-amino-1,2,4-triazole (3AT; Sigma) to prevent background growth. After 5 days at 30°C, colonies were patched on the same medium and replica plated for β-galactosidase activity. To rescue the binding plasmids, HB101 bacterial cells were transformed with DNA from positive clones. The transformed cells were grown in M9 minimal medium supplemented with the appropriate amino acids. To ensure that each colony incorporated only one plasmid, DNA was prepared from colonies of HB101 cells and was subjected to PCR amplification. To obtain a better quality as well as quantity of the DNA from the positive clones, the chosen plasmids were introduced into DH5α cells from which DNA was prepared. To test the specificity of binding of the rescued plasmids, we retransformed L40 cells with the appropriate Vav plasmid or with pLex-Lamin. Sequences of cDNA inserts from positive clones were performed by the chain termination dideoxy sequencing method and analyzed by the FASTA program.

3. Results

To identify proteins that associate with the amino-terminus region of Vav and are important for its activity as a signal transducer and transforming protein, we used the yeast two hybrid system [24]. As a bait, we chose the amino 178 residues (VavY2H$^{1-178}$) which contain the CH domain (residues 3–115) as well as the acidic region (residues 132–176). Yeast L40 cells were co-transformed with VavY2H$^{1-178}$ and a human cDNA of Jurkat T cells hooked to pGAD1318 that carries the activating domain of GAL4. Fifty clones grew on −LWH medium that contains 50 mM 3AT. These clones also produced β-galactosidase, thus indicating that the bait interacted with a protein encoded by the expression library. The positive interactor plasmids were segregated from the bait plasmid following the procedure detailed in Section 2, and several of them were eliminated as they interacted non-specifically with the control plasmid (Lamin). One of the proteins that bound to the VavY2H$^{1-178}$ bait, interactor (112), showed a 100% identity to an already known human protein, Ly-GDI, a 28 kDa protein, that has striking homology to the product of a previously cloned gene, Rho-GDI [25,26]. These proteins were shown to inhibit GDP/GTP exchange on the Rho family of GTPases [25,26,28]. Unlike Rho-GDI, Ly-GDI is expressed only in hematopoietic tissues and predominantly in B and T lymphocyte cell lines [25,26]. It is noteworthy that none of the isolated proteins under the stringent conditions that we used corresponded to ENX-1 or hSoc1.

To assess the nature of the association between Vav and Ly-GDI, we employed the highly sensitive GST in vitro binding assay (Fig. 1A, upper panel). Vav from NIH/proto-vav cells was found to associate specifically with Ly-GDI expressed as a GST bacterial fusion protein (GST-Ly; lane 4), but not with GST (lane 3). No binding to GST or GST-Ly from NIH3T3 cells could be observed (lanes 5 and 6). The ratio of bound Vav protein to GST-Ly (lane 4) versus the amount of Vav present in the cells, as determined by immunoprecipitation with anti-Vav Abs (lane 2), indicates that a substantial amount of Vav binds to Ly-GDI. Similar results were obtained when the blot was rehybridized with anti-Ptyr Abs (Fig. 1A, lower panel).

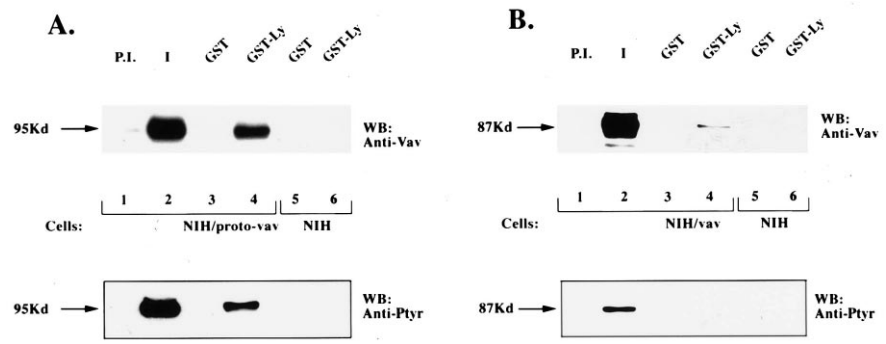

Fig. 1. Association of Vav (A) and the product of the *vav* oncogene (B) with Ly-GDI. (A) Lysates of NIH/proto-vav were immunoprecipitated with either preimmune sera (P.I.; lane 1) or anti-Vav Abs (I; lane 2). In addition, bacterial fusion proteins that express GST (lanes 3 and 5) and Ly-GDI (GST-Ly; lanes 4 and 6) were immobilized on glutathione Sepharose beads and incubated with lysates of NIH/proto-vav cells (lanes 3 and 4) and NIH3T3 (lanes 5 and 6). The bound proteins were resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), immunoblotted with anti-Vav Abs (upper panel) and rehybridized with anti-Ptyr Abs (lower panel). The arrow indicates the position of the product of Vav (95 kDa). (B) A similar experiment to the one described in (A) was performed, except lysates of NIH/vav cells were used instead of lysates of NIH/proto-vav. The arrow indicates the position of the product of the *vav* oncogene (87 kDa).

Since yeast cells co-transformed with either the oncogenic *vav* region (*vavY2H*$^{1-45}$) or the amino residues 1–93 of Vav (Vav2H$^{1-93}$) together with Ly-GDI grew on −LW plates, but failed to grow on −WLH plates (data not shown), we concluded that the amino acid residues critical for *vav* transformation do not directly bind to Ly-GDI. We then tested whether Ly-GDI associates with the product of the *vav* oncogene (87 kDa) which lacks the amino 67 residues that do not associate with Ly-GDI (see above), but retains the rest of the protein. A similar experiment to the one described in Fig. 1A was performed, except NIH/vav cells were used (Fig. 1B, upper panel). Only a tiny fraction of the truncated/transforming form of Vav present in the cells, as evaluated by immunoprecipitation (lane 2), bound to GST-Ly (lane 4). Moreover, the use of anti-Ptyr Abs illustrated that there is no tyrosine-phosphorylated *vav* protein associated with GST-Ly, probably due to the low level of bound protein (Fig. 1B, lower panel). The fact that the *vav* sequences critical for transformation do not associate with Ly-GDI (yeast two hybrid, data not shown) and the truncated/transforming *vav* protein (which retains residues 68–845) hardly associates with Ly-GDI (Fig. 1B) strongly suggest that an intact amino-terminus of Vav is needed for its efficient binding to Ly-GDI.

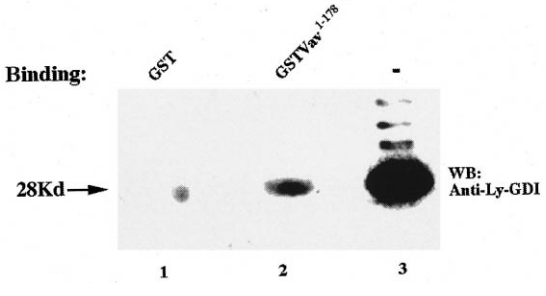

Fig. 2. The product of Ly-GDI associates with Vav. Lysates of Jurkat cells were either incubated with GST (lane 1) and GSTVav$^{1-178}$ (lane 2) bacterial fusion proteins immobilized on glutathione Sepharose beads or loaded unbound on a SDS–PAGE (lane 3). The proteins were resolved on SDS–PAGE and immunoblotted with anti-Ly-GDI. The arrow indicates the position of the product of Ly-GDI (28 kDa).

We then measured the binding of Ly-GDI to the amino region of Vav (residues 1–178) expressed as a GST bacterial fusion protein (GSTVav$^{1-178}$; Fig. 2). Ly-GDI from Jurkat T cells associates with GSTVav$^{1-178}$ (lane 2), but not with GST (lane 1). The relatively small fraction of Ly-GDI bound to GSTVav$^{1-178}$ compared to its level in the cells (lane 2 vs. lane 3) probably reflects an improper folding of the amino-terminus region of Vav when expressed as a GST fusion protein. No binding of Ly-GDI could be demonstrated to either GSTVav$^{1-93}$ or GSTVav$^{66-178}$ (data not shown), thus con-

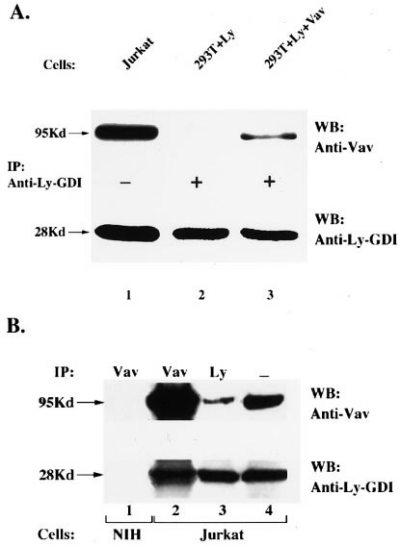

Fig. 3. Vav associates with Ly-GDI in the cells. (A) Lysates of 293T cells that were transfected with either Ly-GDI (293T+Ly; lane 2) or Ly-GDI and Vav (293T+Ly+Vav; lane 3) were immunoprecipitated with anti-Ly-GDI Abs. The immunoprecipitated proteins and lysates of Jurkat T cells (lane 1) were resolved on SDS–PAGE, immunoblotted with anti-Vav Abs (upper panel) and then rehybridized with anti-Ly-GDI Abs (lower panel). (B) Lysates of NIH3T3 (lane 1) and Jurkat T cells (lanes 2 and 3) were immunoprecipitated with anti-Vav Abs (Vav; lanes 1 and 2) or anti-Ly-GDI Abs (Ly; lane 3). The immunoprecipitated proteins and lysates of Jurkat cells (lane 4) were resolved on SDS–PAGE, immunoblotted with anti-Vav Abs (upper panel) and then rehybridized with anti-Ly-GDI Abs (lower panel). The arrows indicate the positions of the product of Vav (95 kDa) and Ly-GDI (28 kDa).

firming that an intact amino-terminus of Vav is needed for maximal binding to Ly-GDI.

We next tested whether the interaction between Vav and Ly-GDI also occurs in the cells (Fig. 3A). Only when 293T cells were transiently transfected with both Vav and Ly-GDI, anti-Ly-GDI Abs co-immunoprecipitated the Vav protein (Fig. 3A, upper panel, lane 3). However, when 293T cells expressed Ly-GDI only (Fig. 3A, lower panel), Vav could not be detected (Fig. 3A, upper panel, lane 2). These results clearly illustrate that the interaction between Ly-GDI and Vav is most likely to occur in vivo. These results were further validated when a similar experiment was performed with Jurkat T cells, in which Ly-GDI and Vav are both physiologically expressed (Fig. 3B). Thus, Vav could be detected when lysates of Jurkat T cells were immunoprecipitated with anti-Ly-GDI Abs (Fig. 3B, upper panel, lane 3) and respectively Ly-GDI is present when lysates of Jurkat T cells were immunoprecipitated with anti-Vav Abs (Fig. 3B, lower panel, lane 2). Again, the interaction between Vav and Ly-GDI is distinctly demonstrated in the cells.

It was previously demonstrated that in Jurkat T cells, stimulation of the TCR by crosslinking with anti-CD3 (OKT3) monoclonal Abs increases tyrosine phosphorylation of a number of proteins, including Vav [5,6]. We therefore analyzed whether Vav from OKT3-stimulated Jurkat T cells will associate preferentially to Ly-GDI (Fig. 4). Our experiments clearly demonstrate that an equal amount of Vav from activated (+; Fig. 4A, lane 6) or non-activated (−; Fig. 4A, lane 5) T cells associates to GST-Ly, despite the fact that an increase in tyrosine phosphorylation of Vav could be observed (Fig. 4B, lane 3 vs. lane 2). Thus, the association between Vav and Ly-GDI does not depend on the tyrosine phosphorylation status of Vav.

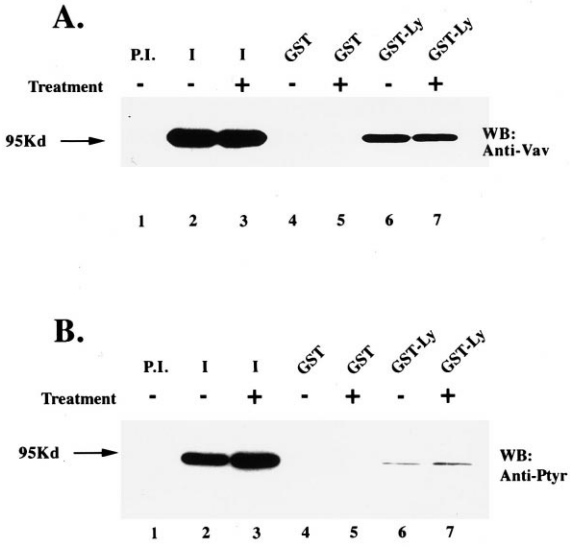

Fig. 4. The association between Vav and Ly-GDI is not dependent on stimulation of the TCR. Lysates of Jurkat T cells that were either OKT3-activated (+) or non-activated (−) were immunoprecipitated with preimmune sera (P.I.; lane 1) or anti-Vav Abs (I; lanes 2 and 3). In addition, lysates of Jurkat T cells either non-activated or OKT3-activated were bound to GST (lanes 4 and 5) or GST-Ly (lanes 6 and 7). The bound/immunoprecipitated proteins were resolved on SDS–PAGE, immunoblotted with anti-Vav Abs (A) and rehybridized with anti-Ptyr Abs (B). The arrow indicates the position of the product of Vav (95 kDa).

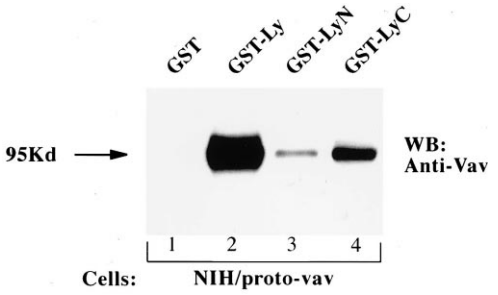

Fig. 5. An intact Ly-GDI exhibits maximal binding to Vav. Lysates of NIH/proto-vav were incubated with GST (lane 1), GST-Ly (lane 2), GST-LyN (lane 3) or GST-LyC (lane 4) immobilized on glutathione Sepharose beads. The bound proteins were resolved on SDS–PAGE and immunoblotted with anti-Vav Abs. The arrow indicates the position of the product of Vav (95 kDa).

The cDNA encoding Ly-GDI that we have isolated through our yeast two hybrid screen contained the entire coding region of the protein (data not shown). Although the structure of Ly-GDI is not yet resolved, the fact that it is highly homologous to Rho-GDI suggests that its structure will be similar. Rho-GDI is composed of two structurally distinct regions of the protein. One is the N-terminal region (residues 1–60) that is flexible and unstructured in solution and is required for inhibition of nucleotide dissociation from Cdc42. The second region is the carboxy-terminus (residues 59–204) that is similar to the structure of an immunoglobulin and is involved in binding to the Rho family member Cdc42, yet it does not affect the rate of nucleotide dissociation from the GTPase [29,30]. We next wished to establish the requirements for binding of Vav to Ly-GDI (Fig. 5). Our results show that the interaction of Vav with either the region in Ly-GDI that is homologous to the flexible N-terminal arm of Rho-GDI (1–59; GST-LyN) or with the immunoglobulin-like domain (residues 60–201; GST-LyC) is reduced (lanes 3 and 4), compared to its binding to the intact Ly-GDI protein (lane 2). These results imply that the entire Ly-GDI is needed for maximal association with Vav. However, the putative immunoglobulin-

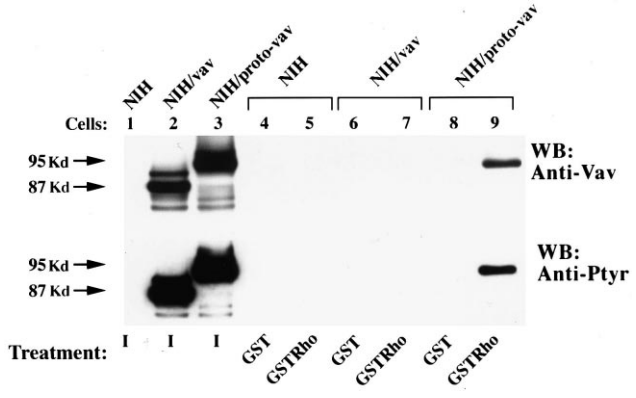

Fig. 6. Association of Rho-GDI with Vav and the product of the *vav* oncogene. Lysates of NIH3T3 (lanes 1, 4 and 5), NIH/vav (lanes 2, 6 and 7) and NIH/proto-vav (lanes 3, 8 and 9) were either immunoprecipitated with anti-Vav Abs (I; lanes 1–3) or bound to either GST (lanes 4, 6 and 8) or GSTRho (lanes 5, 7 and 9). The bound/immunoprecipitated proteins were resolved on SDS–PAGE, immunoblotted with anti-Vav Abs (upper panel) and rehybridized with anti-Ptyr Abs (lower panel). The arrows indicate the positions of Vav (95 kDa) and the product of the *vav* oncogene (87 kDa).

like domain exhibits substantial binding to Vav (lane 3) as compared to the binding properties of the presumed flexible N-terminal arm (lane 4).

Taking into account that the highest homology between Ly-GDI and Rho-GDI is at their carboxy region, which is critical for Ly-GDI interaction with Vav (Fig. 5), and that both GDIs are expressed in Jurkat T cells, we tested whether Rho-GDI associates with Vav (Fig. 6). Our results clearly illustrate that a substantial amount of Vav present in NIH/proto-vav cells (Fig. 6A, lane 3) binds to Rho-GDI when expressed as a GST fusion protein (GSTRho; Fig. 6A, lane 9). This is also verified by the use of anti-Ptyr Abs (Fig. 6B). Moreover, only a minute amount of the truncated/oncogenic *vav* (87 kDa) which could solely be observed after longer exposures of the blot (data not shown and Fig. 6, lane 7) associates with Rho-GDI, despite the fact that this protein is highly expressed in NIH/vav (Fig. 6, lane 2).

4. Discussion

We report here the identification of Ly-GDI as a protein that until recently was an elusive partner of the region of Vav (amino-terminus) that is critical for its activity as a transforming and signal transducing protein. Vav (95 kDa) from Jurkat T cells (where it is exclusively expressed) and from NIH3T3 cells (where it is ectopically expressed) interacts with Ly-GDI both in vitro and in vivo (Figs. 1–5). By contrast, the transforming protein of *vav* (87 kDa) which lacks part of its amino-terminus hardly associates with Ly-GDI (Fig. 1B). These results correlate with the observation that removal of 127, 168 or 186 residues of the amino-terminus of Vav produces a protein that transforms even more potently than the oncogene that we originally isolated [31,32]. The loss of binding that follows amino-terminal truncation or replacement by pSV2neo-derived residues does not imply that this region directly interacts with Ly-GDI (data not shown), but rather suggests that the amino-terminal region of Vav is involved in protein folding and is required for maximal binding of Vav to Ly-GDI.

The question then arises whether additional proteins that bind to Vav are also critical for its biological activity. Truncation of 65 amino acids at the amino-terminus of Vav disrupts the putative actin-binding, CH domain (residues 3–115). Nonetheless, the direct association of Vav with actin through this region has been disputed by Gimona and Winder [33]. In addition, ENX-1 that was reported to associate with the amino-terminus of Vav associates with residues 66–172 of Vav, but not with the region of Vav that is critical for its transforming activity (residues 1–65) [23]. Hence, the association between Vav and ENX-1 may be important for the function of Vav as a signal transducer protein, but is not critical for *vav* transformation.

The fact that Vav binds also to Rho-GDI (which unlike Ly-GDI is expressed in fibroblasts) suggests that this interaction might be sufficient to block Vav transformation, unless its amino-terminus is truncated, and its association with Rho-GDI is disrupted (Fig. 6). Indeed, transformation of NIH3T3 cells by the *vav* proto-oncogene was blocked when Ly-GDI was added in excess, whereas no differences in the transforming potential were exhibited when the *vav* oncogene was used under similar conditions (Groysman et al., unpublished data). In hematopoietic cells, where both GDI proteins (Ly-GDI and Rho-GDI) interact with Vav, more than one GTP-binding protein might be affected. Several studies indicate that Ly-GDI and Rho-GDI might not simply represent redundant proteins, but they probably have more complex and distinctive activities. For instance, knock-out of Ly-GDI in mice leads only to a slight reduction in the capacity of macrophages lacking Ly-GDI to generate superoxide and has no effect on lymphoid or hematopoietic development [34,35]. By contrast, knock-out of Rho-GDI leads to progressive impairment of kidneys and reproductive organs [36]. In addition, although Rho-GDI and Ly-GDI can associate with the same Rho proteins (RhoA, Cdc42H and Rac1) in vitro, these proteins are in vivo partners of Rho-GDI but not of Ly-GDI [37]. Loss of the ability of the truncated Vav protein to stimulate NF-AT activity in T cells [18] is most probably the result of its inability to interact with GDI proteins, an interaction that is most likely required for the proper function of Vav in T cells.

How then could the interaction between Vav and GDI proteins control the biological activity of Vav? Vav stimulates the exchange of GDP to GTP ('on') of GTP-binding proteins such as Rho, Rac and Cdc42H [7,8]. This activity is tightly regulated by its tyrosine phosphorylation status. However, the transforming *vav* protein that lacks the Ly-GDI-binding region is constitutively active without being regulated by tyrosine signaling cascades [7,8]. On the other hand, the GDIs were shown to inhibit the dissociation of GDP from members of the Rho proteins, thus keeping the GTP-binding proteins in a non-active form ('off') [25,26,28,38]. Rho-GDI was shown to strongly prevent the exchange factors from activating the Rho proteins by interacting with its substrate, unless their association is disturbed by proteins such as ezrin/radixin/moesin proteins (ERM) [39,40] or Merilin [41]. We envision that Vav competes with proteins such as ERM for their binding to GDI proteins, thus preventing GDI from interacting with its substrate and permitting the exchange of GDP to GTP ('on') by the DH domain. Alternatively, GDIs could serve as chaperone-like proteins that translocate GTP-binding proteins through the cytosol to their membrane site of action. Loss of the association of oncogenic *vav* with GDIs might enhance its binding to the membrane fraction, where it can be more active as a GDP/GTP exchange factor. This effect may help to explain the results of Abe et al. [32] who concluded that the amino-terminus deletion of Vav led to its enhanced association with the membrane-containing particulate fraction. Which of these hypotheses indeed reflects the intricate situation in vivo remains to be established experimentally. Nonetheless, the association between Vav and GDIs (Ly-GDI and Rho-GDI) likely regulates the activity of Vav as an exchange factor.

In conclusion, Vav and Ly-GDI are proteins that are exclusively expressed in hematopoietic cells. Both are involved in the GDP/GTP exchange processes, one as an exchange factor (Vav) and the other as a dissociation inhibitor factor (Ly-GDI). Moreover, Vav associates with the ubiquitously expressed Rho-GDI. Our results imply that the interaction between Vav and GDIs contributes an additional regulatory step to the role of Vav in signaling cascades following activation of hematopoietic cells and probably also to its function as an oncogene.

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