Identification of the *Drosophila melanogaster* homologue of the mammalian signal transducer protein, Vav

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Abstract Mammalian Vav signal transducer protein couples tyrosine kinase signals with the activation of the Rho/Rac GTPases, thus leading to cell differentiation and/or proliferation. We have isolated and characterized the *DroVav* gene, the homologue of h*Vav* in *Drosophila melanogaster*. *DroVav* encodes a protein (793 residues) whose similarity with hVav is 47% and with hVav2 and hVav3 is 45%. DroVav preserves the unique, complex structure of hVav proteins, including the 'calponin homology', *dbl* homology, pleckstrin homology; SH2 and SH3 domains in addition to regions that are acidic rich, proline rich and cysteine rich. *DroVav* is located on the X chromosome in polytene interval 18A5;18B and is expressed in all stages of development and in all tissues. In mammalian cells, DroVav is tyrosine-phosphorylated in response to epidermal growth factor receptor (EGFR) induction; in vitro, the DroVav SH2 region is associated with tyrosine-phosphorylated EGFR. Thus, DroVav probably plays a pivotal role as a signal transducer protein during fruit fly development.

Key words: Vav; Signal transducer; *DroVav*

1. Introduction

The identification of multiple proteins that participate in tyrosine signal transduction cascades has been greatly facilitated by the discovery of a series of oncogenes that represent aberrant forms of growth factor receptors, growth factors, transducers of growth factor responses and transcription factors [1,2]. The *vav* proto-oncogene (*Vav*) is a cytoplasmic signal transducer that plays a pivotal role in hematopoietic cells where it is exclusively expressed [3–6]. Vav is tyrosine-phosphorylated in response to activation by one of several cytokines, growth factors or antigen receptors [3,4,7,8]. Tyrosine phosphorylation of Vav regulates its activity as a guanine-nucleotide exchange factor for the Rho-like small GTPases RhoA, Rac1 and Cdc42, which lead to cytoskeletal reorganization and activation of stress-activated protein kinases (SAPK/JNKs) [9,10].

The role of Vav in vivo has been studied in a number of ways. Embryonic stem cells homozygous for a null mutation in *Vav* were injected into blastocysts of RAG-1 or RAG-2 deficient mice [11–13]. These manipulations led to a reduction in the various populations of B and T cells normally observed in wild type mice and to defective antigen receptor-mediated proliferation of B and T cells [11–13]. However, T lymphocytes from *Vav* knockout mice respond abnormally to T cell stimulation [5,6]. Both capping of the T cell receptor (TCR) following cell activation and receptor recruitment of the actin cytoskeleton to the CD3 ζ chain of the TCR are defective in mice deficient for *Vav*. So also are interleukin-2 production, cellular proliferation and cell cycle progression, activity of NF-ATc, phosphorylation of SLP-76 and Ca^{2+} mobilization [5,6]. Hence in T cells, Vav integrates signals from lymphocyte antigen receptors and costimulatory receptors to control differentiation, proliferation and response to activation.

Vav is one part of a large family of proteins including hVav2 with which it shares 64% similarity [14,15], and hVav3 with which it shares 71% similarity [16]. The hVav2 and hVav3 proteins contain the characteristic structural motifs known to play a role in tyrosine-mediated signal transduction, found in Vav. These motifs include the following domains: a *dbl* homology domain (DH) that activates GTP-bound proteins of the Rho-like family of proteins [17–19]; a pleckstrin homology (PH) domain [20]; a Src homology 2 (SH2) and two Src homology 3 (SH3) domains [21,22]; a proline rich (PR) motif that allows it to bind to SH3 containing proteins [3]; an acidic rich (AR) region [3]; a 'calponin homology' (CH) region, which functions as an actin-binding domain in other proteins [23] and a cysteine rich (CR) region. Moreover, both hVav2 and hVav3 exhibit biochemical and functional properties similar to those mediated by Vav. However, unlike Vav, Vav2 and Vav3 are also expressed in many tissues/cells of non-hematopoietic origin [14–16]. Thus, the Vav family of proteins is a novel family of signal transducers that couple tyrosine kinase signals with the activation of the Rho/Rac family of GTPases, events that are important in the regulation of cell differentiation and proliferation in most tissues.

There are several cell surface receptor tyrosine kinase (RTK) signaling pathways to the nucleus that have been found to be conserved between mammalians and flies. These conserved pathways have been shown to control differentiation and morphogenesis in various developmental stages of *Drosophila melanogaster* (reviewed in [24,25]). This similarity makes it possible to use flies to analyze the molecular mechanisms involved in signal transduction processes in a way that would not be possible in mammalian cells. Here, we report on the isolation and characterization of the *D. melanogaster Vav* (*DroVav*), and provide initial evidence for its potential role in signaling processes.

2. Materials and methods

2.1. Isolation of DroVav cDNA

An expressed sequence tag (EST) cDNA clone (GenBank accession number AA736164) encoding a polypeptide with 50% homology to

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the carboxy-terminus of h*Vav* was obtained from Research Genetics, and the 1.8 kb cDNA fragment was used to screen a λgt11 *Drosophila* embryonal (8–12 h) cDNA library (kindly provided to us by Dr. T. Volk). Eight positive clones were obtained, of which one was found by PCR analysis to contain the presumptive 5′ end. This *DroVav* clone was digested with *Eco*RI, subcloned in pBluescript KS− and sequenced by an automated sequencer using overlapping oligonucleotides that annealed to both strands (pID11; GenBank accession number AF218780). The alignment of the Vav proteins and structural analysis of conserved motifs was performed by using the European Bioinformatics Institute Clustalw program and the EMBL Simple Modular Architecture Research Tool.

2.2. In situ hybridization to polytene chromosomes

Crawling third instar larvae were fixed, hybridized and stained as previously described [26]. A genomic clone containing the *DroVav* locus was labeled with biotin using the Bioprime DNA Labeling System (Gibco BRL).

2.3. RNA in situ hybridization

Both sense and antisense RNA *DroVav* probes from a cDNA clone including base pairs 1083–1953 bp downstream from the initiation codon were synthesized using T3 and T7 polymerases with a Maxiscript in vitro transcription kit (Ambion). Overnight collections of eggs from wild type and *Df(1)JA27/FMZ,Scr-lacZ* flies were hybridized and detected as described previously [27]. Antisense lacZ probes were also used to distinguish *Df(1)JA27/Y* embryos from those carrying the *FM7,Scr-lacZ* balancer chromosome.

2.4. Reverse transcription (RT-) PCR, Southern and Northern blot analyses

Poly-A RNA was extracted from wild type *D. melanogaster* at various developmental stages with the Poly-A tract 1000 system (Promega). cDNAs were prepared with an oligo-dT primer by using the Superscript II reverse transcriptase (Gibco BRL). The resulting cDNA mixtures were subjected to PCR amplification with the following *DroVav* oligonucleotide primers: 5′-GAGACATCGCCGCTGCAC-3′ and 5′-TCGCGGCCAATAGTGCGT-3′. The PCR products were separated on 1.5% agarose gel, stained with ethidium bromide and transferred to a nylon membrane (0.45 μm) on which they were hybridized with a DIG-labeled *DroVav* probe. The *DroVav* probe employed was a cDNA clone extending from base pair 1083 downstream from the initiation codon to the 3′ poly-A tail. To perform a Northern blot analysis, the poly-A RNAs extracted as described above were resolved on 1% morpholine propanesulfonic acid (MOPS)/formaldehyde agarose gel, transferred onto a nylon membrane (Nytrane 0.45 μm) on which they were hybridized to the ^{32}P-labeled *DroVav* cDNA probe described above.

2.5. Bacterial fusion proteins

A DroVavSH2 glutathione-*S*-transferase (GST) fusion protein was generated by a PCR-based methodology, using the full length *DroVav* plasmid DNA (pID11) as a template. The 5′ oligonucleotide contained a *Bam*HI restriction enzyme site (underlined): 5′-GGCGGGGGATCCAATTGGTTTGCGGGCAAC-3′ and the 3′ oligonucleotide contained an *Eco*RI restriction enzyme site (underlined): 5′-CCGGGGGAATTCCACCTCCTTGTAGGGCCA-3′. The PCR product was digested with *Bam*HI and *Eco*RI restriction enzymes and subcloned into the pGEX-2TK vector.

2.6. Mammalian expression vectors

h*Vav* was cloned at the *Eco*RI site of the mammalian expression vector driven by the CMV promoter, pCDNA3, thus generating pCS114. Full length *DroVav* was generated by PCR amplification by using the following primers: 5′-CCGGGGTCTAGAATGGCCAGCAGCAGT-3′ and 5′-CCGGGGCTCGAGAAGCTCTTCGCTGGC-3′. The PCR product was digested with *Xba*I/*Xho*I (underlined above) and subcloned at the *Nhe*I/*Xho*I sites of pSecTagA (Invitrogen), a mammalian expression vector driven by the CMV promoter that contains a Myc epitope tag (pID18).

2.7. Binding of cell lysate proteins to bacterial GST fusion proteins immobilized on glutathione Sepharose beads

Cell lysates were incubated with the appropriate fusion proteins immobilized on glutathione Sepharose beads (5 μg), resolved on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immunoblotted with antibodies (Abs) as described previously [7,28].

2.8. Cell culture

NIH3T3 murine fibroblasts that overexpress the epidermal growth factor receptor (EGFR) (HER14; [29]) and HEK293 were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum.

2.9. Gene transfer assays

HEK293 cells were transiently transfected with 10 μg DNA of various plasmid DNAs (see Section 3) by using FUGENE 6 (Boehringer Mannheim). The efficiency of transfection was confirmed by the fluorescence of the green fluorescence plasmid included in the transfection.

2.10. EGF stimulation

HEK293 cells transfected with various mammalian expression vectors and HER14 cells grown to subconfluence were starved in DMEM containing 5 μg/ml insulin and 5 μg/ml transferrin for 24 h. Cells were then treated with DMEM containing 0.5 μg/ml human EGF at 37°C for 2 min, washed twice with phosphate-buffered saline and lysed as described above.

2.11. Abs

Monoclonal anti-phosphotyrosine (Ptyr) Abs, 4G10, anti-Myc Abs used to detect the Myc-tagged DroVav and anti-Vav Abs were purchased from UBI.

3. Results and discussion

We initially screened the Berkeley *D. melanogaster* Genome Project (BDGP) ESTs databank for cDNA clones similar to h*Vav*. Our search led to the identification of an EST clone (HL08101) that contains a 1.8 kb insert whose sequence corresponds to the carboxy-terminus of h*Vav*. To isolate the entire coding region of the *D. melanogaster* homologue of human *Vav* (*DroVav*), we screened a *D. melanogaster* cDNA library with the 1.8 kb insert of HL08101 as a probe. The longest cDNA clone isolated, pID11, contained an open reading frame of 2379 nucleotides with an ATG that conforms with the consensus translation/initiation codon, a 490 bp 5′ untranslated region (UTR) and a 1.2 kb 3′ UTR. A further search of GenBank with the predicted DroVav protein sequence identified hVav as the most closely related protein. Examination of pID11 revealed that it encoded a protein of 793 amino acid residues, which shares 47% similarity with hVav and 45% similarity with both hVav2 and hVav3 (Fig. 1). An alignment of the domain structure of *DroVav* with hVav, hVav2 and hVav3 reveals that *DroVav* preserves the complex structure found in hVav proteins. These include a CH domain, an AR region, a DH domain, a PH domain, PR sequence, CR region, a SH2 and a SH3 domain (Fig. 1). These results strongly suggest that we have indeed isolated the *Drosophila* homologue of *Vav* (*DroVav*).

The Vav family of proteins has a unique and highly characteristic structure that includes numerous defined known functional regions [3,4,14–16]. In particular no other protein or protein family includes the DH, PH, SH3 and SH2 regions. Among the signal proteins in *D. melanogaster*, the structure of DroVav is unique. One other *Drosophila* protein, DrtGEF, is known to include the DH, PH and SH3 regions [30]; however, it lacks the SH2 domain that is shared by DroVav and hVav.

Though there is a great deal of homology between the DroVav and hVav proteins, they are not identical: DroVav lacks the hVav amino-SH3 domain (SH3N). Sequence alignment

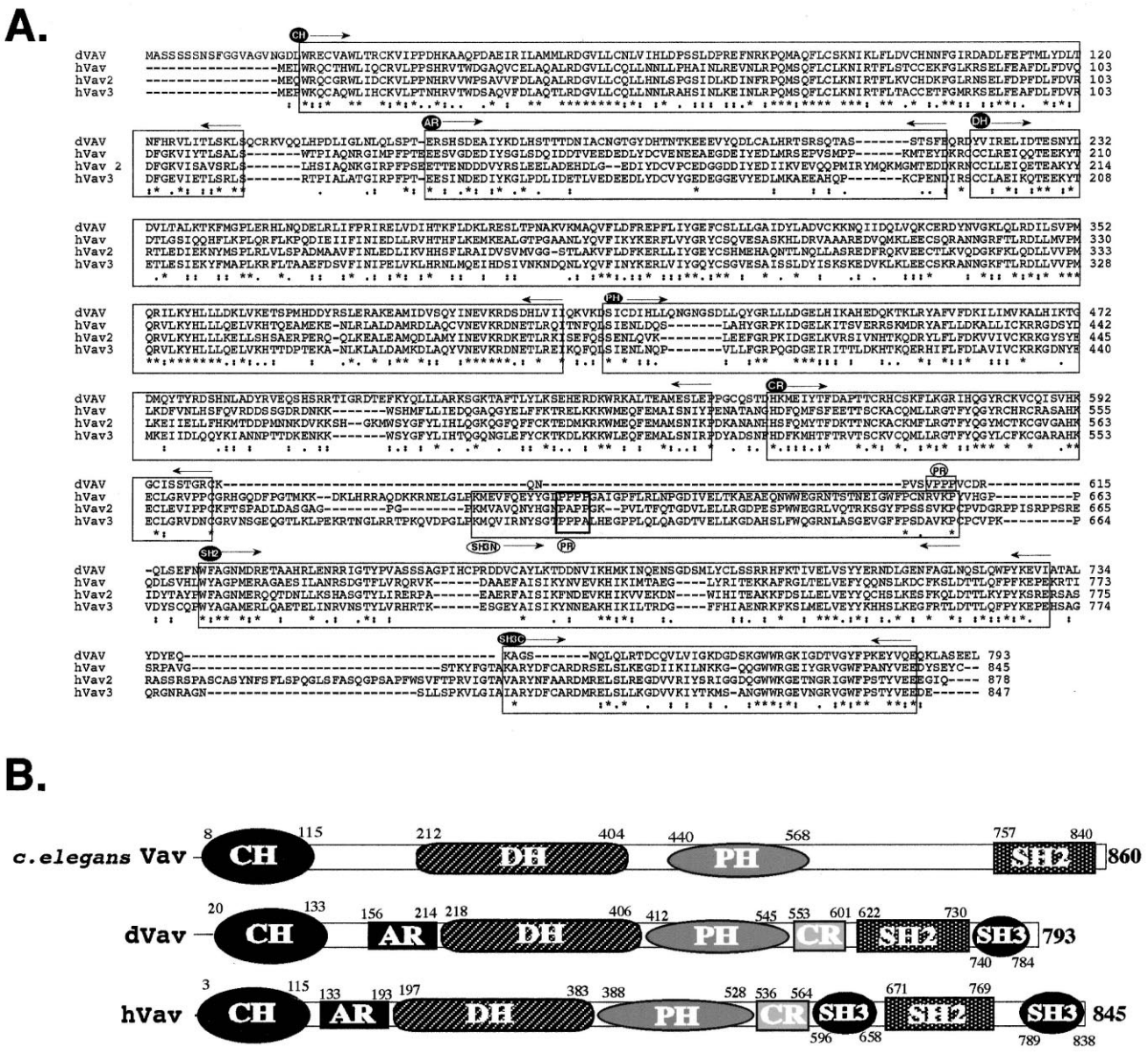

Fig. 1. Comparison of amino acid residues of DroVav with hVav, hVav2 and hVav3 (A) and schematic representation of the domain structure of Vav from *C. elegans*, *D. melanogaster* and humans (B). (A) The amino acid sequence of DroVav (dVav) is aligned with the three known members of the hVav family of proteins. Symbols below the alignment represent: '*' for identical residues; ':' for conserved residues and '.' for semi-conserved residues. The individual structural domains are boxed. Each domain/motif is also marked by arrows that designate the start and end to the region in addition to the contraction of each region: CH, a 'calponin homology'; AR, an acidic rich region; DH, a *dbl* homology domain; PH, a pleckstrin homology domain; CR, cysteine rich; PR, proline rich; SH2, Src homology 2; and SH3, Src homology 3. Shaded boxes represent structural domains absent in one or more of the proteins aligned. (B) Schematic representation of the domain structure of *C. elegans* Vav, DroVav (dVav) and hVav. The numbers correspond to the amino acid boundaries of each domain.

between the two SH3 regions in the hVav polypeptide reveals that the SH3 structure at the hVavSH3N differs from that at its carboxy-terminal end, hVavSH3C. As we have previously reported, the hVavSH3C domain is more active in binding proline rich proteins than is the hVavSH3N domain [31]. Sequence alignment of the amino acid residues of the Vav homologue in *Caenorhabditis elegans* (*C. elegans* Vav) with that of hVav and DroVav (Fig. 1B) revealed that the CH, DH, PH and SH2 regions are conserved, but that both SH3 regions are missing. It is therefore conceivable that it were specifically the SH3 regions that evolved in the development of the protein from *C. elegans* Vav to human Vav. Thus, the analysis of the domain structures of the Vav proteins from various species of increasing evolutionary complexity should highly be indicative of evolutionary modifications. Moreover, the identification of *Vav* in species of different evolutionary complexity might be highly suggestive of its pivotal role in signaling cascades.

Several of the conserved tyrosines in the human Vav proteins are also found in DroVav (Fig. 1), including the hVav Tyr^{142} (DroVav Tyr^{145}) and the hVav Tyr^{209} (DroVav Tyr^{231}). Note that the target for phosphorylation by Src or Syk family protein tyrosine kinases, resulting in activation of its exchange activity, hVav Tyr^{174} [10], is not found in DroVav (Fig. 1A). It was recently demonstrated that Tyr^{174} is not required for the TCR-induced phosphorylation of Vav in vivo, but rather that a mutation in this residue leads to gain in the ability of

Vav to up-regulate NF-AT activation [32,33]. These results suggest that tyrosine residues additional to Tyr^{174} may also play a critical role in the function of Vav proteins. Most of the tyrosines in hVav that can be phosphorylated remain at present unmapped. The fact that several of the conserved tyrosines in the hVav proteins are also found in DroVav might facilitate mapping and analyzing them.

To determine whether *DroVav* locus is involved in any of the known mutations in *D. melanogaster*, we used a genomic clone containing *DroVav* to localize it to polytene chromosomes. In situ hybridization showed that the *DroVav* locus is located toward the base of the X chromosome in interval 18A5;18B (Fig. 2A). To find potential mutations in the *DroVav* locus, we searched the *D. melanogaster* database (flybase.bio.indiana.edu) and found two deletion mutants, *Df(1)N19* (17A1;18A2) and *Df(1)JA27* (18A5;18D), from which parts of the 18A interval were missing. We repeated the in situ hybridization to polytene chromosomes heterozygous for the deletion chromosomes and a wild type chromosome. *DroVav* failed to hybridize to *Df(1)JA27* chromosomes while it hybridized to wild type homologues (Fig. 2B) and to *Df(1)N19* chromosomes (not shown). We conclude that *Df(1)JA27* deletion mutation removes the *DroVav* locus.

Human Vav proteins are signal transducers that are either highly restricted in their tissue distribution, like hVav that is exclusively expressed in the hematopoietic system [3], or are widely distributed like hVav2 and hVav3 [14–16]. To determine the in vivo pattern of expression of *DroVav* in *D. melanogaster*, we performed RNA in situ hybridization on *D. melanogaster* embryos (Fig. 3A). An overnight collection of eggs from wild type and *Df(1)JA27/FMZ,Scr-lacZ* flies was hybridized with sense and antisense *DroVav* probes. *DroVav* RNA was found abundantly in 0–2 h embryos, prior to the start of zygotic transcription (Fig. 3A, a). High levels of ubiquitous *DroVav* RNA could also be detected following the initiation of zygotic transcription (Fig. 3A, b–d). The level of *DroVav* was greatly reduced in embryos that were

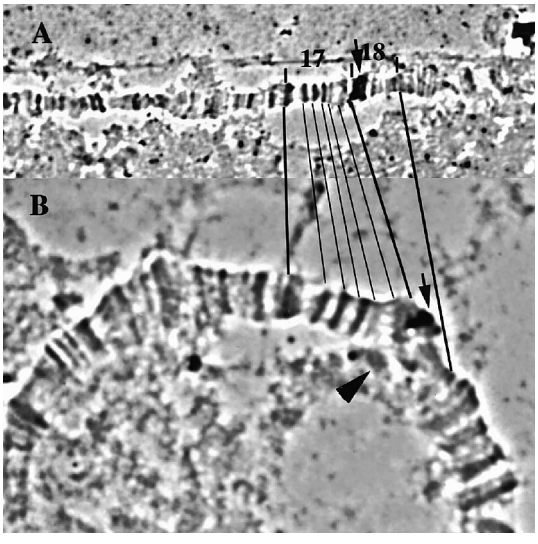

Fig. 2. In situ hybridization to polytene chromosomes localizes *DroVav* to the X chromosome. Crawling third instar larvae were fixed, hybridized and stained as previously described [26]. A genomic clone containing the *DroVav* locus was labeled with biotin and hybridized with *DroVav* (A; arrow) and *Df(1)JA27* chromosome (B; arrowhead).

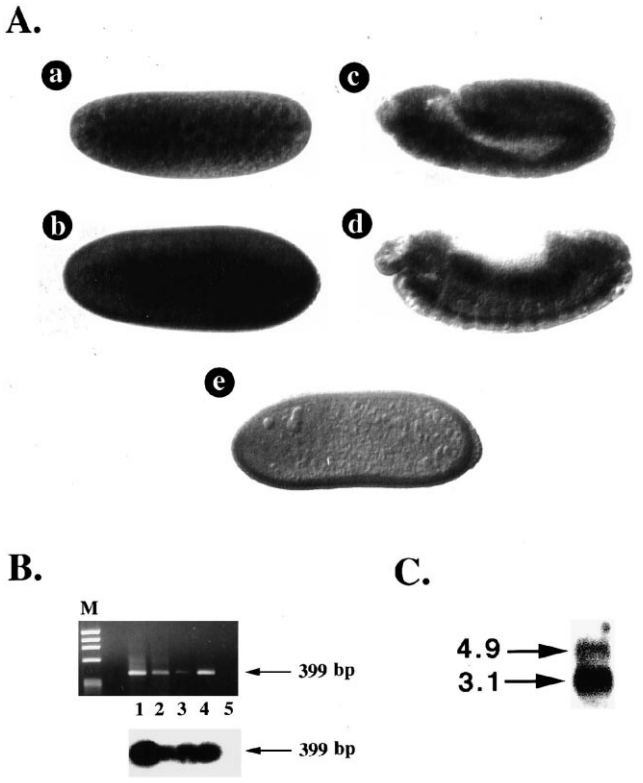

Fig. 3. Developmental expression (A), distribution (B) and size (C) of the DroVav transcript. (A) Antisense RNA *DroVav* probe was hybridized in situ with embryos of the following stages: early cleavage stage prior to the start of zygotic transcription (a), during cellular blastoderm (b), germ band extension (c) and germ band retraction (d) stages. In addition, embryos lacking the *DroVav* locus, *Df(1)JA27*, were also used (e). (B) RT-PCR (upper panel) and Southern blot analysis (lower panel) was performed on poly-A RNA extracted from wild type (wt) *Drosophila* embryos (0–24 h; lane 1), third instar larvae (lane 2), adult males (lane 3) and adult females (lane 4) by using *DroVav* primers. The PCR product, encompassing nucleotides 1083–1482 (399 bp indicated by an arrow) from the initiation codon, was separated on 1.5% agarose gel and stained with ethidium bromide (B, upper panel). The reaction was also performed on a mixture without DNA (lane 5). The *DroVav* RT-PCR products were then transferred to a nylon membrane which was hybridized with a DIG-labeled *DroVav* cDNA probe (B, lower panel). Lane M refers to size marker ϕX174 digested with *Hae*III. (C) Northern blot analysis was performed on poly-A RNA extracted from adult females, resolved on 1% MOPS/formaldehyde agarose gel, transferred onto a nylon membrane and hybridized to a ^{32}P-labeled *DroVav* cDNA probe. Similar results were obtained with RNA extracted from embryos (0–24 h), third instar larvae and adult males (data not shown).

Df(1)JA27 deletion mutants and hence lacked the *DroVav* locus (Fig. 3A, e). These embryos were identified by their failure to hybridize to an antisense lacZ probe expressed from the *FMZ,Scr-lacZ* chromosome (data not shown). In addition, *DroVav* RNA was not detected in approximately 1/4 of the embryos analyzed, the same frequency expected for *Df(1)JA27/Y* embryos. There is also a large maternal contribution to embryonic *DroVav*. Using the *Df(1)JA27* deficiency embryos, we have shown that maternal RNA is present. This RNA disappears by the gastrulation stage. To test whether DroVav is expressed in additional developmental stages, we performed a RT-PCR analysis (Fig. 3B, upper panel); our results indicated that *DroVav* is present in embryos

(0–24 h; lane 1), in third instar larvae (lane 2), and in both adult males (lane 3) and females (lane 4). Southern blot analysis verified that the amplified fragment corresponds to *DroVav* (Fig. 3B, lower panel). Northern blot analysis revealed two species of *DroVav* transcripts of 3.1 and 4.9 kb that we found in embryos (a 0–24 h collection), in third instar larvae, and in both adult males and females (Fig. 3C and data not shown). The 3.1 kb transcript was found to be the major one expressed as compared to the expression level of the 4.9 kb transcript (Fig. 3C and data not shown). The 4.9 kb minor transcript may represent either an alternatively spliced form of *DroVav* or another unidentified related Vav signal transducer protein. We are currently investigating these possibilities.

Together, our results showed that there are high levels of *DroVav* expression in all embryonic tissues and at all subsequent stages of *D. melanogaster* development. This suggests that DroVav may function in various signaling pathways in *D. melanogaster* as do the hVav proteins in humans. The particular in vivo spatial and temporal patterns of *DroVav* function are yet to be identified.

A characteristic hallmark of Vav signal transducer proteins is that they become phosphorylated on tyrosine residues in response to EGF stimulation [7,34,35]. Furthermore, Vav proteins are known to bind to the stimulated EGFR through their SH2 region [7,34,35]. Since the encoded domain structure of DroVav strongly suggests that it may function as a signal transducer protein, we tested its response to EGF stimulation (Fig. 4). HEK293 cells were transiently transfected with either an empty mammalian expression vector (pSec; Fig. 4A, lanes 1 and 2), *DroVav* (Fig. 4A, lanes 3 and 4) or h*Vav* (Fig. 4A, lanes 5 and 6). We observed tyrosine phosphorylation of DroVav (lane 4) and hVav (lane 6) only when the HEK293 cells had been stimulated with EGF (+; Fig. 4A, upper panel). No tyrosine phosphorylation of these proteins could be observed when the cells were non-induced (−; Fig. 4A, upper panel, lanes 3 and 5). Yet, comparable levels of DroVav (Fig. 4A, middle panel, lane 3 vs. 4) and hVav (Fig. 4A, lower panel, lane 5 vs. 6) were present in both the non-induced and induced cells. Thus, we conclude that DroVav (92 kDa) is tyrosine-phosphorylated following the induction of signaling processes by EGF. Next, we tested whether DroVav could associate with the stimulated EGFR through its SH2 region. Fig. 4B illustrates that following incubation with lysates of EGF-induced (+) HER14 cells, a 170 kDa tyrosine-phosphorylated protein associated with DroVavSH2 (Fig. 4B, lane 4) and with hVavSH2 (Fig. 4B, lane 6), both expressed as GST bacterial fusion proteins. By immunoblotting with EGFR Abs, this 170 kDa was confirmed to be the EGF receptor (data not shown). The binding of EGFR to hVavSH2 is stronger than to DroVavSH2, probably due to species-specific differences. These results indicate that the SH2 region of DroVav can associate with tyrosine-phosphorylated proteins like EGFR.

We have shown that when DroVav is expressed in mammalian cells, its tyrosine residues become phosphorylated in response to EGF stimulation (Fig. 4A). Furthermore, the SH2 region of DroVav can associate with the phosphorylated EGFR (Fig. 4B). These two results are highly compatible with our earlier observations regarding hVav [7,34], and thus strongly suggest that DroVav probably functions as a signal transducer protein. Nevertheless, though our results have not yet established DroVav as a target protein in

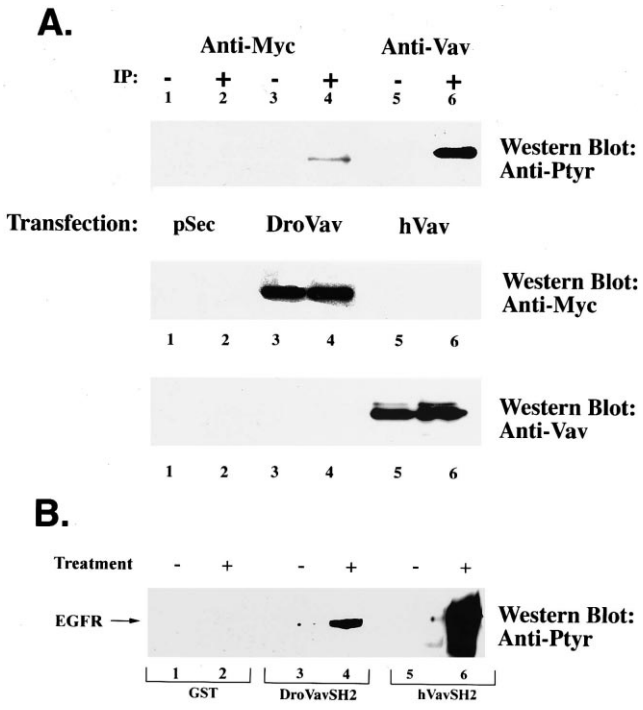

Fig. 4. Tyrosine phosphorylation of DroVav (A) and association of DroVavSH2 with tyrosine-phosphorylated EGFR (B). (A) HEK293 cells that were transfected with either pSecTagA (pSec; lanes 1 and 2), DroVav (pID18; lanes 3 and 4) or hVav (pCS114; lanes 5 and 6) were either stimulated with EGF (+; lanes 2, 4 and 6) or non-stimulated (−; lanes 1, 3 and 5). Lysates of these cells were immunoprecipitated with either anti-Myc Abs (lanes 1–4) or anti-Vav Abs (lanes 5 and 6). The immunoprecipitated proteins were resolved on SDS–PAGE, immunoblotted with anti-Ptyr Abs (upper panel) and then rehybridized with either anti-Myc Abs (middle panel) or anti-Vav Abs (lower panel). (B) Bacterial fusion proteins that express GST (lanes 1 and 2), the SH2 region of DroVav (DroVavSH2; lanes 3 and 4) and the SH2 of hVav (hVavSH2; lanes 5 and 6) were immobilized on glutathione Sepharose beads and incubated with lysates of cells overexpressing human EGFR (HER14) that were either non-induced (−; lanes 1, 3 and 5) or induced (+; lanes 2, 4 and 6) with 0.5 μg/ml EGF for 2 min. The proteins were then resolved on SDS–PAGE and immunoblotted with anti-Ptyr Abs.

EGFR signaling cascades, they do suggest that the function of DroVav is similar to that of hVav. This conclusion is further strengthened by the fact that *D. melanogaster* RTK cascades, consisting of signaling molecules like the EGF receptor DER [36], and the GRB2 homologue DRK [37], are highly conserved with their mammalian counterparts, where they have been shown to interact with human Vav proteins [7,34,38]. Moreover, the *D. melanogaster* homologues to the Rho-like small GTPases (RhoA, Rac1 and Cdc42) have been identified [39–41] and found to be similar to the mammalian Vav-regulated Rho proteins [9,10]. These small GTPases have been shown to be activators of the *D. melanogaster* JNK pathway, part of the control system of developmental pathways like embryonic dorsal closure [42,43] and muscular and neuronal development [39]. Based on all these observations, we predict that a high degree of conservation will be found between DroVav and hVav pathways.

Because there are several members in the Vav family of proteins in humans, it is difficult to differentiate between possibly redundant and/or more complex functions. The fact that the Vav genes have probably evolved from one ancestral gene

together with the highly conserved and unique structure of DroVav suggests that it is an important regulatory molecule in the life of the cell/organism. Our studies of Vav in a lower organism like the fruit fly *D. melanogaster* provide a powerful tool to examine its function and to determine its role in signaling cascades.

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