Vav1 Oncogenic Mutation Inhibits T Cell Receptor-induced Calcium Mobilization through Inhibition of Phospholipase Cγ1 Activation

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Background: The oncogenic version of Vav1 inhibits calcium signaling in T cells.
Results: Oncogenic Vav1 alters the fine structure of the signaling clusters and inhibits phospholipase Cγ1 (PLCγ1).
Conclusion: Oncogenic mutation of Vav1 impairs Vav1 adaptor functions and interferes with PLCγ1 activation.
Significance: These findings shed new light on the mechanisms underlying the adaptor-like functions of Vav1 and the effect produced by its oncogenic mutation.

Robust elevation of the cytosolic calcium concentration is a crucial early step for T cell activation triggered by the T cell antigen receptor. Vav1 is a proto-oncogene expressed in hematopoietic cells that is indispensable for transducing the calcium-mobilizing signal. Following T cell receptor stimulation, Vav1 facilitates formation of signaling microclusters through multiple interactions with other proteins participating in the signaling cascade. Truncation of the N terminus of Vav1 produces its oncogenic version, which is unable to support normal calcium flux following T cell activation. We show here that truncation of the N-terminal region of Vav1 alters the fine structure of protein complexes in the signaling clusters, affecting the interaction of Vav1 with phospholipase Cγ1 (PLCγ1). This alteration is accompanied by a decrease in PLCγ1 phosphorylation and inhibition of inositol 1,4,5-trisphosphate production. We suggest that the structural integrity of the N-terminal region of Vav1 is important for the proper formation of the Vav1-associated signaling complexes. The oncogenic truncation of this region elicits conformational changes that interfere with the Vav1-mediated activation of PLCγ1 and that inhibit calcium mobilization.

T lymphocytes are an integral and indispensable part of the immune system and participate in a vast majority of immunological responses. Some T cells produce cytokines, which direct and regulate responses of other members of the immune system. Other T cells are responsible for seeking out and killing tumor cells or cells infected with viruses. Compromised function of T lymphocytes results in severe, often fatal immunodeficiencies or autoimmune diseases.

T cell activation begins when the T cell antigen receptor (TCR) is stimulated by an antigen. Proximal events that immediately follow TCR engagement include activation of protein kinases and phosphorylation of multiple enzymes and adaptor molecules (1–4). These events result in formation of intricate and highly dynamic protein complexes, which activate multiple signaling pathways leading to dramatic alterations in lymphocyte gene expression as well as profound changes in cellular shape and motility. These complexes have been visualized using fluorescence microscopy techniques and have become known as signaling microclusters (5–10).

Robust elevation of the cytosolic Ca2+ concentration ([Ca2+]i) is a crucial early step for T cell activation triggered by the TCR. The amplitude and duration of this rise in intracellular Ca2+ determine the strength and form of the immune response (11–15). As in most electrically non-excitable cells, [Ca2+]i elevation in T cells exhibits a typical biphasic pattern: Ca2+ release from intracellular stores, followed by Ca2+ influx from the extracellular medium. Phospholipase Cγ1 (PLCγ1) catalytic activity results in formation of inositol 1,4,5-trisphosphate (IP3). The latter binds to IP3 receptors (IP3Rs) in the endoplasmic reticulum and induces the release of Ca2+ into the cytosol. The depletion of Ca2+ from intracellular stores triggers entry of Ca2+ across the plasma membrane via calcium release-activated calcium channels. It should be noted that although calcium release-activated calcium influx provides the main source of cytosolic Ca2+ in stimulated T cells, its activation and continuation are contingent on an effective depletion of intracellular Ca2+ stores and keeping the stores in a depleted state for an extended period of time.

Recruitment and activation of PLCγ1 in T cells are mediated, among other events, through its interaction with the Rho family GTPase exchange factor (GEF) Vav1 (16, 17). Vav1 is a 95-kDa protein expressed in hematopoietic cells, contains multiple domains characteristic of signal-transducing proteins, and asserts its effects through both GEF-dependent and GEF-independent functions (see Fig. 1A) (18–20). The GEF activity of Vav1 is dispensable for TCR-induced Ca2+ mobilization, which presumably relies on the adaptor-like functions of Vav1 (21, 22). Being recruited to the TCR-proximal protein complexes upon T cell activation, Vav1 facilitates formation of signaling...
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microclusters through multiple interactions with other proteins participating in the signaling cascade (23–26).

The N-terminal region of Vav1 (~124 amino acids) forms a calponin homology (CH) domain. Together with the acidic domain, it creates an autoinhibitory loop via intramolecular interactions with the Dbl homology domain and the pleckstrin homology domain, which negatively regulates the GEF activity of Vav1 (19, 27). Truncations of the N terminus produce oncogenic versions of Vav1 (oncoVav1) possessing various transformation capacities due to constitutive GEF activity (19, 28, 29).

Vav1-deficient T cells show severely impaired antigen receptor signaling (16, 24, 30–32). Overexpression and reconstitution studies have demonstrated that N-terminally truncated oncoVav1 can rescue many defects produced by Vav1 deficiency, but it is unable to support normal Ca2+ flux and NF-AT activation (19, 26, 30, 33, 34). Thus, in addition to its autoinhibitory role, the CH domain has an important stimulatory function in T cells. Specifically, this domain is necessary for TCR-induced Ca2+ flux. However, the mechanism underlying this critical function of the CH domain of Vav1 remains a mystery. So far, few proteins have been reported to interact with the CH domain of Vav1, and the significance of these interactions for Ca2+ signaling remains unclear (35–37).

We hypothesize that the CH domain truncation affects the scaffolding properties of Vav1, disrupting some of its interactions within the TCR-proximal signaling complexes. Several known binding partners of Vav1, e.g. PLCγ1, Itk, Slp76, and LAT, have been implicated in mediating Ca2+ signaling in T cells (17, 38–43). Interestingly, none of these proteins have been shown to interact with the CH domain of Vav1, yet this domain may not necessarily bind the critical downstream transducer itself, but rather contribute to stabilization of interactions via other binding domains of Vav1.

Using advanced microscopy techniques, we show here that truncation of the CH domain indeed alters the fine structure of protein complexes in the signaling clusters; specifically, the interaction of Vav1 with PLCγ1 is affected. This alteration is accompanied by a decrease in PLCγ1 phosphorylation and inhibition of IP3 production. Therefore, we suggest that the structural integrity of the N-terminal region of Vav1 is important for the proper formation of the Vav1-associated signaling complexes. The oncogenic truncation of this region elicits conformational changes that interfere with the Vav1-mediated activation of PLCγ1 and that inhibit TCR-induced calcium mobilization.

EXPERIMENTAL PROCEDURES

Antibodies—Rabbit anti-Vav1 antibody was obtained from Cell Signaling Technology. Anti-GFP antibody was from Roche Applied Science. HRP-conjugated goat anti-mouse IgG and goat anti-rabbit IgG were from KPL. Anti-CD3 antibody HIT3a and Alexa Fluor 647-conjugated anti-CD3 antibody were from BioLegend. Anti-PLCγ1, anti-phospho-PLCγ1 (Tyr-783), and anti-GAPDH antibodies were from Epitomics. Mouse anti-CD3ε antibody OKT3 was provided by Dr. L. E. Samelson (NCI, National Institutes of Health, Bethesda, MD).

Expression Vectors and Plasmids—The human Vav1 cDNA was provided by Dr. D. D. Billadeau (Mayo Clinic College of Medicine, Rochester, MN). The oncoVav1 construct was created by deletion of fragment 1–67 from Vav1. The monomeric YFP plasmid was created from pEYFP-N1 (Clontech) by A206K substitution (44). The Yep construct was provided by Prof. R. S. Daugherty (University of California, Santa Barbara, CA). The Cerulean CFP plasmid was created from pECFP-N1 (Clontech) by A206K, S72A, Y145A, and H148D substitutions (44, 45). The resultant YFP and CFP variants were used to create all fluorescent conjugates by standard methods. LAT-YFP, Slp76-YFP, and PLCγ1-YFP were described previously (10, 17, 46, 47).

Mutations were introduced using a QuikChange II XL site-directed mutagenesis kit (Stratagene). The constructs were cloned into either pMSCVhyg or pMSCVneo (Clontech) to create retroviral expression vectors. All constructs were verified by DNA sequencing.

IP3 Measurements—The FRET-based IP3-sensitive fluorescent probe was created based on the previously described analogs (48–50). The binding region of rat IP3R3(1–604) was obtained from Prof. Yoshio Tojyo (University of Hokkaido, Hokkaido, Japan). The IP3R3(224–579) fragment was flank with Cerulean CFP (henceforth CFP) and the Yep derivative of YFP (henceforth YFP) (see Fig. 3A). The nuclear export sequence LSEALLQLOF derived from NF-kB RelA was added to the N-terminal region of the probe (51). The R504Q mutation was introduced into the IP3R3 fragment to increase probe sensitivity (49). A modified version of the probe containing a loss-of-function K508A mutation in the IP3-binding region was used as a negative control. The functional probe and its negative control version are termed CFY(+) and CFY(−), respectively. Cells were transfected with either CFY(+) or CFY(−) using Amaxa Nucleofector (Lonza) and an Ingenio electroporation kit (Mirus). The cells were used 24 h post-transfection. Uniform cytosolic distribution of the probe was verified by confocal microscopy. The probe fluorescence signal was monitored by flow cytometry using a FACSCan II system (BD Biosciences). Only live cells exhibiting both CFP and YFP signals of comparable strength and a linear correlation between them were used for calculating IP3 changes. This restriction allowed rapid real-time estimation of relative changes in FRET efficiency as described under “Results.”

Vector Expression—E6.1 and J.Vav1 Jurkat cells were maintained in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum. All recombinant DNA constructs, except CFY(+) and CFY(−), were introduced into the cells using retroviral infection. The expression vectors and pVSV-G (Clontech) were cotransfected into GP2–293 packaging cells (Clontech) using a ViraPack transfection kit (Stratagene). After 48 h, the virus-containing medium was removed from the packaging cells and mixed with Jurkat cells. The medium was replaced with the regular growth medium after 24 h, and an appropriate selection medium was added 72 h post-infection. Protein expression was monitored at multiple time points using flow cytometry and Western blotting. TCR expression levels were monitored using immunostaining with Alexa Fluor 647-conjugated anti-CD3 antibody, followed by flow cytometry.

Immunoblotting—Cells were either stimulated with anti-CD3 antibody OKT3 for 2 min or left untreated and lysed in ice-cold lysis buffer (1% Brij 98, 1% sodium deoxycholate, 0.5 M...
Oncogenic Vav1 Inhibits TCR-induced PLCγ1 Activity—The TCR triggers Ca2+ release from intracellular stores via activation of PLCγ1. Phosphorylation of Tyr-783 is necessary for PLCγ1 activation. Therefore, we examined whether PLCγ1 phosphorylation at Tyr-783 is affected by oncoVav1. As evident from Fig. 2, PLCγ1 Tyr-783 phosphorylation was inhibited in Vav1-deficient cells and fully restored in J.Vav1 cells reconstituted with WT Vav1. However, it remained partially attenuated in J.Vav1-oncoVav1 cells. Because the significance of this partial attenuation for the outcome of PLCγ1 activation and IP3 production was unclear, we decided to assess the extent of the TCR-induced PLCγ1 activity by monitoring changes in cytosolic levels of IP3.

To evaluate the changes in IP3 levels induced by TCR stimulation, we constructed a FRET-based IP3-sensitive fluorescent probe based on the previously described analogs (48–50). The fragment of the IP3R IP3-binding domain was flanked with the Cerulean derivative of CFP and the YPet derivative of YFP (Fig. 3A). To exclude the probe from the nucleus, which takes up most of the T cell volume, a putative nuclear export sequence (51) was added to the N-terminal region of the probe. The uniform cytosolic expression of the probe and its exclusion from cell nuclei were verified using fluorescence microscopy (data not shown). A modified version of the probe containing a loss-of-function K508A mutation in the IP3-binding region was used as a negative control (49). The functional probe and its negative control version are termed CFY(+) and CFY(−), respectively.

The working principle of this and similar probes is based on the assumption that binding of IP3 brings up a conformational change that alters the distance between the probe fluorophores and consequently the FRET efficiency exhibited by them. The changes in FRET efficiency exhibited by the IP3 probe following TCR stimulation were estimated by illuminating the probe-expressing cells with the CFP excitation laser line and calculating the ratio between the fluorescence intensity obtained from the YFP emission window (FRET channel) and the CFP emission window (CFP channel), i.e. $F_{\text{ratio}} = F_{\text{FRET}}/F_{\text{CFP}}$. By restricting the collected data to cells exhibiting CFP and YFP fluorescence of comparable intensities and linear correlation between those intensities, this method allowed rapid real-time approximation.
of relative changes in FRET efficiency without the need for elaborate calculations required to obtain pure FRET fluorescence intensities and absolute FRET efficiency values.

According to Fig. 3 (B–F), both E6.1 and J.Vav1-WT cells expressing CFY(+) exhibited a clearly observable elevation in \( F_{\text{ratio}} \) in response to the TCR stimulation, yet no detectable change in \( F_{\text{ratio}} \) was observed in E6.1 and J.Vav1-WT cells expressing CFY(–). Thus, the observed elevation in \( F_{\text{ratio}} \) is indicative of an increase in IP\(_3\) levels, and the probe can indeed be used for monitoring relative changes in intracellular IP\(_3\) concentrations. In addition, neither J.Vav1 nor J.Vav1-oncoVav1 cells expressing CFY(+) showed any detectable change in \( F_{\text{ratio}} \). In fact, their responses were virtually indistinguishable from those of cells expressing CFY(–).

These results suggest that IP\(_3\) production is inhibited in J.Vav1-oncoVav1 cells to a similar extent as in J.Vav1 cells. Thus, the detrimental effect of oncoVav1 on TCR-induced Ca\(^{2+}\) flux is most likely a result of an impaired PLC\(\gamma\)1 activity.

**Oncogenic Mutation of Vav1 Interferes with Vav1/PLC\(\gamma\)1 Interaction within Signaling Clusters**—The primary function of Vav1 in the process of TCR-induced Ca\(^{2+}\) mobilization is that of an adaptor protein (21, 22). Upon TCR stimulation, Vav1 is recruited to signaling complexes, where it interacts with multiple signaling proteins, including PLC\(\gamma\)1 (17, 38–43). To visualize recruitment of Vav1 following TCR stimulation, we created fluorescent versions of WT Vav1 and oncoVav1 by fusing them with CFP. The resulting constructs were expressed in J.Vav1 cells. To verify that the fluorescently labeled proteins
retained their effects on calcium signaling during T cell activation, the calcium assay described above (Fig. 1A) was performed again using reconstituted J.Vav1 cells, this time with the fluorescent analogs of WT Vav1 and oncoVav1. As evident from Fig. 4, the fluorescently labeled proteins (Vav1-CFP and oncoVav1-CFP) performed similarly to their unlabeled counterparts (compare Figs. 1A and 4). Interestingly, CFP-Vav1, the N-terminally labeled version of WT Vav1, failed to restore calcium mobilization in J.Vav1 cells. Therefore, only the C-terminally labeled variants (Vav1-CFP and oncoVav1-CFP) were used in further experiments.

To evaluate whether the oncogenic mutation of Vav1 affects recruitment of Vav1 to signaling clusters and its interactions with other signaling proteins, we coexpressed either Vav1-CFP or oncoVav1-CFP with LAT-YFP, Slp76-YFP, or PLCγ1-YFP. These three proteins were chosen because they are recruited to the signaling clusters, interact with Vav1 within the clusters, and are critical mediators of TCR-induced Ca²⁺ mobilization (17, 38–41). Vav1-CFP and oncoVav1-CFP were expressed at levels comparable to the level of Vav1 expression in E6.1 cells (Fig. 4B). The YFP-fused proteins were expressed at levels equal to or moderately exceeding the expression levels of CFP-fused proteins to create optimal conditions for FRET-based experiments (Fig. 4C).

Our imaging analysis was based on a previously published technique (10, 17, 47). In this protocol, T cells are dropped onto a surface coated with a stimulatory monoclonal antibody that binds the TCR. This results in TCR clustering and recruitment of multiple signaling molecules to the points of contact with the stimulatory surface within 1–3 min (10, 17, 47). Similar clusters of signaling molecules have been observed upon T cell contact with lipid bilayers containing antigen-MHC complexes (9).

The results shown in Fig. 5 demonstrate that both Vav1-CFP and oncoVav1-CFP co-localized completely with LAT-YFP, Slp76-YFP, and PLCγ1-YFP. No detectable difference in the distribution pattern of Vav1-CFP and oncoVav1-CFP was
observed. Therefore, the oncogenic mutation of Vav1 does not interfere with its recruitment to the signaling clusters. However, we have shown previously that the recruitment is necessary but may not be sufficient for normal function of signaling proteins within the cluster; thus, interference with selected interactions within the signaling complex results in nonproductive recruitment of PLCγ1 (17).

We have demonstrated previously that the FRET efficiency measured between fluorescently labeled signaling proteins is strongly sensitive to the structural variations within the signaling clusters (17, 47). Thus, comparing FRET efficiency values observed between either fluorescently labeled Vav1 or oncVav1 and their binding partners might point to structural abnormalities within the signaling clusters created by the oncogenic mutation of Vav1.

As shown in Fig. 5 (A and B), no significant FRET occurred between LAT-YFP and either Vav1-CFP or oncVav1-CFP. Thus, this assay cannot provide additional insight into the effect of the oncogenic mutation of Vav1 on the interaction of Vav1 with LAT.

On the other hand, Slp76-YFP exhibited a strong FRET signal with Vav1-CFP (Fig. 5C). However, this FRET signal was not significantly altered by the oncVav1-CFP substitution (Fig. 5D). Therefore, the interaction of Vav1 with Slp76 is probably not impaired by the oncogenic mutation of Vav1. PLCγ1-YFP also exhibited substantial FRET efficiency with Vav1-CFP (Fig. 5E). However, unlike Slp76-YFP, this FRET signal was essentially abolished with oncVav1-CFP (Fig. 5F). The statistical analysis of FRET results is summarized in Fig. 6.

Taken together, these results suggest that truncation of the CH domain of Vav1 alters the fine structure of the TCR-induced signaling complex. Although Vav1 does not bind PLCγ1 via its CH domain, the structural changes induced by the CH domain truncations are sufficient to interfere with the Vav1/PLCγ1 interaction. This leads to inhibition of the TCR-induced PLCγ1 activity, IP3 production, and Ca2+ mobilization.
**DISCUSSION**

The proto-oncogene vav1 plays crucial roles in the process of T cell activation (18–20). Upon TCR stimulation, Vav1 is recruited to the TCR-proximal protein complexes, where it serves as a scaffold for other signaling proteins within the complexes (23–26). The recruitment of Vav1 to the signaling clusters is essential, among other effects, for TCR-induced Ca\(^{2+}\) mobilization (16, 24, 30–32). On the other hand, CH domain-truncated oncoVav1 is also recruited to the signaling clusters following TCR stimulation but fails to mediate Ca\(^{2+}\) mobilization.

We have shown here that oncoVav1 does not compromise the capacity of intracellular calcium stores, nor does it impede Ca\(^{2+}\) influx triggered by calcium store depletion (Fig. 1C). These results are consistent with previously reported findings (52). It has also been reported that PLC\(\gamma\)1 phosphorylation is not significantly altered by oncoVav1 expression (52). However, our results indicate that the TCR-induced phosphorylation of the critical Tyr-783 residue of PLC\(\gamma\)1 is diminished in cells expressing oncoVav1 compared with cells expressing WT Vav1 (Fig. 2).

To further assess the effect of oncoVav1 on PLC\(\gamma\)1 activity, we created and implemented a FRET-based fluorescent probe for monitoring changes in cytosolic IP\(_3\) levels. The ability of the probe to detect changes in IP\(_3\) concentration was evaluated via comparison between F\(_{\text{ratio}}\) values obtained from CFP-labeled WT Vav1 (Fig. 1A) and CFP-labeled oncoVav1 (Fig. 1B). The results shown in Fig. 3 indicate that IP\(_3\) production in oncoVav1-expressing cells was inhibited.
FIGURE 5. FRET analysis between either WT Vav1-CFP or oncoVav1-CFP and various YFP-labeled proteins. J.Vav1 cells expressing either WT Vav1-CFP (A, C, and E) or oncoVav1-CFP (B, D, and F) with LAT-YFP (A and B), Slp76-YFP (C and D), or PLCγ1-YFP (E and F) were seeded on a stimulatory coverslip and fixed 2 min into the activation process. FRET efficiency (FRETeff) values are presented in a pseudo-colored scale, with black representing saturated pixels. The average value of FRET efficiency ± S.E. obtained for a given pair is shown. The FRET efficiency values for each pair have been calculated from at least 10 separate fields containing one to three cells each. Size bars = 5 μm.
compared with WT Vav1-expressing cells and resembled the IP3 production levels obtained in the Vav1-deficient J.Vav1 cells. Therefore, although PLCγ1 Tyr-783 phosphorylation was only partially inhibited by oncoVav1, PLCγ1 activity was substantially reduced in oncoVav1-expressing cells. It is possible that in addition to reducing the efficiency of PLCγ1 Tyr-783 phosphorylation, oncoVav1 interferes with the ability of PLCγ1 to attain and/or maintain its active conformation following phosphorylation.

Recently performed structural studies indicate that the CH domain truncation induces extensive conformation changes in the Vav1 molecule (53, 54). We have demonstrated previously that the FRET efficiency measured between fluorescently labeled signaling proteins is strongly sensitive to the structural variations within the TCR-proximal signaling clusters (17, 47). Therefore, we have employed the fluorescent imaging and FRET approach to evaluate the possible effect of the CH domain truncation on the structure of the Vav1-containing protein complexes. Our results shown here, together with results obtained by others (34), indicate that the CH domain truncation does not impair recruitment of Vav1 to the signaling clusters, albeit moderately affecting the persistence and mobility of the clusters. However, the profound change in FRET efficiency values observed between Vav1 and PLCγ1 when WT Vav1 was replaced with oncoVav1 suggests that the internal structure of the complex is distorted by the Vav1 CH domain truncation. Specifically, the relative position and/or orientation of Vav1 and PLCγ1 within the complex is altered by the truncation. We have shown previously that efficient activation of PLCγ1 within the TCR-proximal signaling complexes requires interaction of multiple signaling proteins under stringent structural conditions (17). Thus, distortion of the signaling complex structure induced by the CH domain truncation might be directly responsible for the oncoVav1-dependent inhibition of PLCγ1 activation.

The accumulated body of evidence suggests that productive recruitment of Vav1 to the signaling clusters is critically reliant on a specific conformation of the Vav1 molecule. Moreover, the
“closed” GEF-autoinhibited conformation most probably mediates PLCγ1 activation. The following evidence points to this conclusion. Truncation of the CH domain, which inhibits Ca2+ mobilization, results in a profound conformational change resembling the “open” GEF-active conformation (53, 54). The GEF activity of Vav1 is dispensable for TCR-induced Ca2+ mobilization (21, 22, 34). Phosphorylation of three tyrosine residues within the acidic domain of Vav1, which triggers transition to the open conformation, is also not required for Ca2+ mobilization. Moreover, mutation of these three residues to phenylalanine augments TCR-induced Ca2+ mobilization (33, 34). In addition to the CH domain truncation, three point mutations of Vav1 that block Ca2+ mobilization have been identified: N74A, L213A, and L278Q (33, 34). These residues are important for creation of the interdomain interfaces formed in the closed conformation of Vav1 (34, 53, 54).

To summarize, we have show here that the oncogenic truncation of the CH domain of Vav1 inhibits the TCR-stimulated PLCγ1 activity. The CH domain truncation affects the structure of the Vav1-containing signaling clusters, altering the position/orientation of Vav1 relative to PLCγ1 within the clusters. We propose that the TCR-induced activation of PLCγ1 is mediated by Vav1 in its closed conformation, whereas the conformational changes triggered by the CH domain truncation, which destabilizes the closed conformation, impair Vav1 adapter-like functions within the PLCγ1-containing signaling complexes and interfere with PLCγ1 activation.

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REFERENCES

1. Simeoni, L., Smida, M., Posevitiz, V., Schraven, B., and Lindquist, J. A. (2005) Right time, right place: the organization of membrane-proximal signaling. Semin. Immunol. 17, 35–49
2. Samelson, L. E. (2002) Signal transduction mediated by the T cell antigen receptor: the role of adaptor proteins. Annu. Rev. Immunol. 20, 371–394
3. Seminario, M. C., and Bunnell, S. C. (2008) Signal initiation in T cell receptor microclusters. Immunol. Rev. 221, 90–106
4. Fooksman, D. R., Vardhana, S., Vasiliver-Shamis, G., Liese, J., Blair, D. A., Waite, J., Sacristán, C., Victora, G. D., Zanin-Zhorov, A., and Dustin, M. L. (2010) Functional anatomy of T cell activation and synapse formation. Annu. Rev. Immunol. 28, 79–105
5. Balagopalan, L., Barr, V. A., and Samelson, L. E. (2009) Endocytic events in TCR signaling: focus on adaptors in microclusters. ImmunoL Rev. 232, 84–98
6. Billadeau, D. D. (2010) T cell activation at the immunological synapse: new mechanisms and functions in T cell development and activation. J. Biol. Chem. 285, 284–287
7. Purrbueh, M. A., Liu, H., Oddos, S., Owen, D. M., Neal, M. A., Pageon, S. V., French, P. M., Rudd, C. E., and Davis, D. M. (2010) Dynamics of subsynaptic vesicles and surface microclusters at the immunological synapse. Sci. Signal. 3, ra36
8. Saito, T., Yokosuka, T., and Hashimoto-Tane, A. (2010) Dynamic regulation of T cell activation and co-stimulation through TCR microclusters. FEBS Lett. 584, 4865–4871
9. Varma, R., Campi, G., Yokosuka, T., Saito, T., and Dustin, M. L. (2006) T cell receptor-proximal signals are sustained in peripheral microclusters and terminated in the central supramolecular activation cluster. Immunity 25, 117–127
10. Bunnell, S. C., Hong, D. I., Kardon, J. R., Yamazaki, T., McClade, C. J., Barr, V. A., and Samelson, L. E. (2002) T cell receptor ligation induces the formation of dynamically regulated signaling assemblies. J. Cell Biol. 158, 1263–1275
11. Oh-hora, M., and Rao, A. (2008) Calcium signaling in lymphocytes. Curr. Opin. Immunol. 20, 250–258
12. Lewis, R. S. (2007) The molecular choreography of a store-operated calcium channel. Nature 446, 284–287
13. Feske, S. (2007) Calcium signaling in lymphocyte activation and disease. Nat. Rev. Immunol. 7, 690–702
14. Gallo, E. M., Canté-Barrett, K., and Crabtree, G. R. (2006) Lymphocyte calcium signaling from membrane to nucleus. Nat. Immunol. 7, 25–32
15. Randriamampita, C., and Trautmann, A. (2004) Ca2+ signals and T lymphocytes: new mechanisms and functions in Ca2+ signaling. Biol. Cell 96, 69–78
16. Reynolds, L. F., Smyth, L. A., Norton, T., Freshney, N., Downward, J., Kioussis, D., and Tybulewicz, V. L. (2002) Vav1 transduces T cell receptor signals to the activation of phospholipase Cγ1 via phosphoinositide 3-kinase-dependent and -independent pathways. J. Exp. Med. 195, 1103–1114
17. Braiman, A., Bara-Saad, M., Sommers, C. L., and Samelson, L. E. (2006) Recruitment and activation of PLCγ1 in T cells: a new insight into old domains. EMBO J. 25, 774–784
18. Katzav, S., Martin-Zanca, D., and Barbacid, M. (1989) vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. EMBO J. 8, 2283–2290
19. Zugaza, J. L., López-Lago, M. A., Caloca, M. J., Dosil, M., Movilla, N., and Bustelo, X. R. (2002) Structural determinants for the biological activity of Vav proteins. J. Biol. Chem. 277, 45377–45392
20. Kuhne, M. R., Xu, G., and Weiss, A. (2000) A guanine nucleotide exchange factor-independent function of Vav1 in transcriptional activation. J. Biol. Chem. 275, 2185–2190
21. Saveliev, A., Vanes, L., Ksionda, O., Rapley, J., Smerdon, S. J., Rittinger, K., and Tybulewicz, V. L. (2009) Function of the nucleotide exchange activity of Vav1 in T cell development and activation. Sci. Signal. 2, ra83
22. Miletic, A. V., Graham, D. B., Sakata-Sogawa, K., Hiroshima, M., Hamann, M. J., Cemerski, S., Kloeppel, T., Billadeau, D. D., Kanagawa, O., Tokunaga, M., and Swat, W. (2009) Vav links the T cell antigen receptor to the actin cytoskeleton and T cell activation independently of intrinsic guanine nucleotide exchange activity. PLoS ONE 4, e6599
23. Tybulewicz, V. L. (2005) Vav family proteins in T cell signaling. Curr. Opin. Immunol. 17, 267–274
24. Turner, M., and Billadeau, D. D. (2002) Vav proteins as signal integrators for multisubunit immune recognition receptors. Nat. Rev. Immunol. 2, 476–486
25. Miletic, A. V., Sakata-Sogawa, K., Hiroshima, M., Hamann, M. J., Gomez, T. S., Ota, N., Kloeppel, T., Kanagawa, O., Tokunaga, M., Billadeau, D. D., and Swat, W. (2006) Vav1 acidic region tyrosine 174 is required for the formation of T cell receptor-induced microclusters and is essential in T cell development and activation. J. Biol. Chem. 281, 38257–38265
26. Wu, J., Katzav, S., and Weiss, A. (1995) A functional T cell receptor signaling pathway is required for p95vav activity. Mol. Cell. Biol. 15, 4337–4346
27. Aghazadeh, B., Lowry, W. E., Huang, X. Y., and Rosen, M. K. (2000) Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogenic Vav by tyrosine phosphorylation. Cell 102, 625–633
28. Schueler, K. E., Movilla, N., Rosa, J. L., and Bustelo, X. R. (1998) Phosphorylation-dependent and constitutive activation of Rho proteins by wild-type and oncogenic Vav2. EMBO J. 17, 6608–6621
29. Katzav, S., Cleveland, J. L., Heslop, H. E., and Pulido, D. (1991) Loss of the amino-terminal helix-loop-helix domain of the proto-oncogene vav induces its transforming potential. Mol. Cell. Biol. 11, 1912–1920
30. Cao, Y., Janssen, E. M., Duncan, A. W., Altman, A., Billadeau, D. D., and Abraham, R. T. (2002) Pleiotropic defects in TCR signaling in a Vav1-null Jurkat T cell line. EMBO J. 21, 4809–4819
31. Costello, P. S., Walters, A. E., Mee, P. J., Turner, M., Reynolds, L. F., Prisco, A., Sarner, N., Zamoyska, R., and Tybulewicz, V. L. (1999) The Rho family GTPase exchange factor Vav is a critical transducer of T cell receptor signals to the calcium, ERK, and NF-κB pathways. Proc. Natl. Acad. Sci. U.S.A. 96, 3035–3040
32. Fujikawa, K., Miletic, A. V., Alt, F. W., Faccio, R., Brown, T., Hoog, J., Fredericks, J., Nishi, S., Mildiner, S., Moores, S. L., Brugge, J., Rosen, F. S.,
Oncogenic Vav1 Inhibits Phospholipase Cγ1

O'Day, K., Schieven, G. L., Lin, T. A., and Kanner, S. B. (2002) Phosphorylation of the linker for activation of T cells by Itk promotes recruitment of Vav. Biochemistry 41, 10732–10740.

Zacharias, D. A. (2002) Sticky caveats in an otherwise glowing report: oligomerizing fluorescent proteins and their use in cell biology. Sci. STKE 2002, pe23.

Rizzo, M. A., Springer, G. H., Granada, B., and Piston, D. W. (2004) An improved cyan fluorescent protein variant useful for FRET. Nat. Biotechnol. 22, 445–449.

Balagopalan, L., Barr, V. A., Sommers, C. L., Bara-Saad, M., Goyal, A., Isakowitz, M. S., and Samelson, L. E. (2007) c-Cbl-mediated regulation of LAT-nucleated signaling complexes. Mol. Cell. Biol. 27, 8622–8636.

Bara-Saad, M., Braiman, A., Titerence, R., Bunnell, S. C., Barr, V. A., and Samelson, L. E. (2005) Dynamic molecular interactions linking the T cell antigen receptor to the actin cytoskeleton. Nat. Immunol. 6, 80–89.

Remus, T. P., Zima, A. V., Bossuyt, J., Bare, D. J., Martin, J. L., Blatter, L. A., Bers, D. M., and Mignery, G. A. (2006) Biosensors to measure inositol 1,4,5-trisphosphate concentration in living cells with spatiotemporal resolution. J. Biol. Chem. 281, 608–616.

Sato, M., Ueda, Y., Shibuya, M., and Umezawa, Y. (2005) Locating inositol 1,4,5-trisphosphate in the nucleus and neuronal dendrites with genetically encoded fluorescent indicators. Anal. Chem. 77, 4751–4758.

Tanimura, A., Nezu, A., Morita, T., Turner, R. J., and Tojo, Y. (2004) Fluorescent biosensor for quantitative real-time measurements of inositol 1,4,5-trisphosphate in single living cells. J. Biol. Chem. 279, 38095–38098.

Harhaj, E. W., and Sun, S. C. (1999) Regulation of RelA subcellular localization by a putative nuclear export signal and p50. Mol. Cell. Biol. 19, 7088–7095.

Zhou, Z., Yin, J., Dou, Z., Tang, J., Zhang, C., and Cao, Y. (2007) The calponin homology domain of Vav1 associates with calmodulin and is prerequisite to T cell antigen receptor-induced calcium release in Jurkat T lymphocytes. J. Biol. Chem. 282, 23737–23744.

Yu, B., Martins, I. R., Li, P., Amasinghe, G. K., Umetani, J., Fernandez-Zapico, M. E., Billadeau, D. D., Machius, M., Tomchick, D. R., and Rosen, M. K. (2010) Structural and energetic mechanisms of cooperative autoinhibition and activation of Vav1. Cell 140, 246–256.

Llorca, O., Arias-Palomo, E., Zugaza, J. L., and Bustelo, X. R. (2005) Global conformational rearrangements during the activation of the GDP/GTP exchange factor Vav3. EMBO J. 24, 1330–1340.