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New insights into the Vav1 activation cycle in lymphocytes

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ABSTRACT

Vav1 is a hematopoietic-specific Rho GDP/GTP exchange factor and signaling adaptor. Although these activities are known to be stimulated by direct Vav1 phosphorylation, little information still exists regarding the regulatory layers that influence the overall Vav1 activation cycle. Using a collection of cell models and activation-mimetic Vav1 mutants, we show here that the dephosphorylated state of Vav1 in nonstimulated T cells requires the presence of a noncatalytic, phospholipase Cγ1–Slp76-mediated inhibitory pathway. Upon T cell stimulation, Vav1 becomes rapidly phosphorylated via the engagement of Lck and, to a much lesser extent, other Src family kinases and Zap70. In this process, Lck, Zap70 and the adaptor protein Lat contribute differently to the dynamics and amplitude of the Vav1 phosphorylated pool. Consistent with a multiphosphosite activation mechanism, the optimal stimulation of Vav1 can only be recapitulated by the combination of several activation-mimetic phosphosite mutants. The analysis of these mutants has also unveiled the presence of different Vav1 signaling competent states that are influenced by phosphosites present in the N- and C-terminal domains of the protein.

1. Introduction

Vav1 mainly works as a tyrosine phosphorylated-regulated Rho guanosine nucleotide exchange factor (GEF), a catalytic activity that allows the rapid transition of Rho GTPases from the inactive (GDP-bound) to the active, GTP-bound state during cell signaling [1,2]. In addition, it displays in some contexts adaptor functions that allow the regulation of downstream signals using catalysis-independent mechanisms [1,3]. For example, Vav1 can promote the Cbl-b-mediated degradation of the intracellular domain of Notch1 [4,5] and the Ca^{2+}-dependent stimulation of the nuclear factor of activated T-cells (NFAT) [6–9], a transcriptional factor essential for the expression of cytokines and other activation-connected proteins in lymphocytes [10]. These adaptor functions can be dependent (NFAT) or independent (Notch1) of the phosphorylation state of Vav1 [1,3–5].

Vav1 is characterized by a multidomain structure that harbors calponin homology (CH), acidic (Ac), Dbl homology (DH), pleckstrin homology (PH), C1-subtype zinc finger (C1), proline-rich (PRR), SH3, and SH2 regions [1] (Fig. 1A). These domains play roles related to the intramolecular regulation of the protein (CH, Ac, PH, SH3) [1,11–14], the activation step (SH2, SH3) [15–17], the catalytic process (DH, PH, C1) [13,14,18,19], the establishment of interactions with protein partners (PRR, SH3, SH2), and the catalysis-independent regulation of NFAT (CH) and Notch1 (the two SH3s) [1,6,8,9,14,20–22]. Whereas the basis of the regulation of the catalytic activity of Vav proteins is well characterized at the structural level [18,19], the mechanism of stimulation of the NFAT route by Vav1 is still poorly understood. However, it is known that it involves the stimulation of phospholipase Cγ (PLCγ) which, upon the IP_3-mediated mobilization of Ca^{2+} from intracellular stores, favors the stimulation of the phosphatase calcineurin by Ca^{2+}-calmodulin [9]. This phosphatase promotes in turn the dephosphorylation of cytoplasmic NFAT and its final shuttling to the nucleus (Fig. 1B). Unlike the case of the catalysis-dependent pathways, the NFAT route requires the parallel action of Vav1 and other TCR-stimulated signaling pathways to achieve full activation in cells [6] (Fig. 1B). As a result, this pathway can be further stimulated by the TCR even

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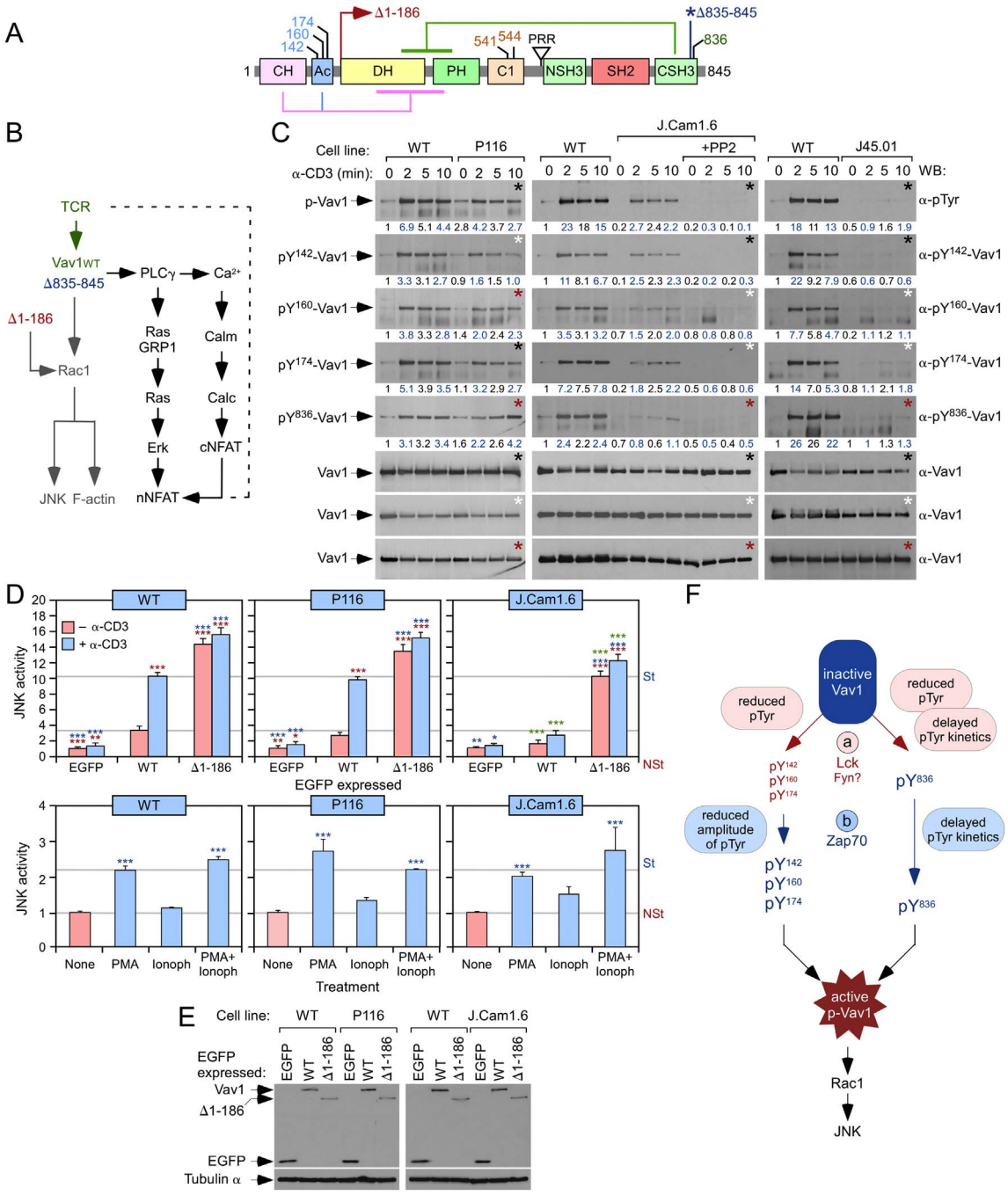

Fig. 1. Tyrosine kinases involved in the Vav1 phosphorylation step.

(A) Vav1 structure indicating phosphosites and intramolecular interactions contributing to Vav1 activity regulation. Phosphosites involved in each intramolecular interaction are depicted in the same color. The truncated N- (Δ1–186) and C-terminal (Δ835–845) mutants used in this study are also indicated.

(B) Simplified view of the Vav1-dependent Rac1-JNK (gray) and NFAT (black) pathways present in lymphocytes [3]. RasGRP1, Ras guanosine nucleotide releasing protein 1 (a diacylglycerol regulated Ras GEF); Calm, calmodulin; Calc, calcineurin; cNFAT, cytosolic NFAT; nNFAT, nuclear NFAT.

(C) Vav1 immunoprecipitates from indicated cells and stimulation conditions were subjected to Western blot (WB) analyses with antibodies shown on the right. Filters were then stripped and reblotted with antibodies to Vav1 (the same filter is indicated with asterisks of the same colour). At the bottom of panels from phosphorylation detection, we present the densitometry values obtained for each immunoprecipitate (given an arbitrary value of 1 to the phosphorylation levels of Vav1 in nonstimulated WT cells). These values were normalized considering the total amount of immunoprecipitated Vav1 obtained in each sample. Please, note that the values can be only compared in each panel and not between panels (given that they were subjected to different exposure times). p-, phospho.

(D) *Top panels,* JNK activation levels in indicated EGFP-expressing Jurkat cell lines (top) that were either left nonstimulated (−) or stimulated (+) with antibodies to CD3. Values are shown as means ± SEM from four independent experiments, each performed in triplicate. *P* values are given relative to nonstimulated (red asterisks) and stimulated (blue asterisks) cells expressing EGFP-Vav1^{WT} in the same cell line. We also included *P* values for the values exhibited by each Vav1 protein relative to those obtained in WT cells (green asterisks). In this latter case, we have not included statistics in mock-transfected cells for the sake of simplicity. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. NSt, nonstimulated; St, stimulated. *Bottom panels*, JNK activation levels in indicated Jurkat cell lines (top) upon stimulation with the indicated compounds (bottom). Ionoph, ionophore. Values and *P* values are given as in top panels.

(E) Example of the abundance of ectopically expressed Vav1 obtained in one the experiments used for panel D.

(F) Summary of the results obtained in this figure. Lck- and Zap70-dependent steps are depicted in red and blue colour, respectively. The defects induced by the elimination of each kinase are boxed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)