Vav1 Couples T Cell Receptor to Serum Response Factor-dependent Transcription via a MEK-dependent Pathway*

Received for publication, December 6, 2001, and in revised form, February 11, 2002
Published, JBC Papers in Press, February 21, 2002, DOI 10.1074/jbc.M111627200

Céline Charvet†§, Patrick Aubéger†, Sophie Tartare-Deckert†, Alain Bernard‡, and Marcel Deckert**

From †INSERM U343, IFR50, Hôpital de l’Archet, 06202 Nice Cedex 3 and ‡INSERM U526 and ¶INSERM U385, IFR50, Faculté de Médecine, Avenue de Valombrose, 06107 Nice Cedex 2, France

The Vav family of guanine nucleotide exchange factors for Rho family GTPases plays a critical role in lymphocyte proliferation, gene transcription, and cytoskeleton reorganization following immunoreceptor stimulation. However, its role in immediate early gene activation is unclear. In this study, we have investigated the mechanisms by which Vav1 can regulate c-fos serum response element transcriptional activity. We show that T cell antigen receptor (TCR) stimulation induces the phosphorylation of serum response factor (SRF) on serine 103 and increases the binding of SRF complexes on serum response element in a MEK- and p38-dependent pathway. The physiological relevance of our findings is supported by the inhibition of the interleukin-2 gene transcriptional activity by a dominant negative SRF mutant. Overexpression of Vav1, which partially mimics TCR stimulation, promotes SRF-dependent transcription, and dominant negative Vav1 mutants block SRF activation by TCR. SRF activation by Vav1 occurs through a signaling cascade consisting of Rac1/Cdc42 and the serine/threonine kinases Pak1 and MEK, but independently of the phosphatidylinositol 3-kinase pathway. Interestingly, Vav2 also enhances SRF through Rho GTPases, suggesting that Vav proteins are general regulators of SRF activation in lymphocytes. This report establishes Vav proteins as a direct link between antigen receptors and SRF-dependent early gene expression.

Antigen receptor engagement on resting T cells activates several signaling pathways, resulting in transcriptional activation of a large number of genes. Within minutes of antigenic stimulation, a complex network of signal transducers enhances the transient transcription of early genes, which in turn regulate a second phase of nuclear events essential for T cell survival, proliferation, differentiation, effector function, and cytokine release (1, 2). Among these early genes, c-fos plays a critical role in diverse physiological processes, including lymphocyte activation. In particular, the binding of Fos and Jun proteins to AP-1 sites is essential for the transcription of several lymphokines and other gene products regulating the immune response (3).

Mitogen-induced c-fos transcription depends essentially on the cis-acting serum response element (SRE)† on its promoter because mutation of the SRE sequence impairs c-fos induction by diverse signals. SRE is also important for the transcriptional induction of other early genes like egr-1. Two transcription factors, the serum response factor (SRF) and the ternary complex factor (TCF), bind to the SRE and promote the transcription of the c-fos promoter and other SRE-regulated genes (4). SRF is ubiquitously expressed and binds as a dimer to the CarG box of the SRE sequence (5). SRF is composed of a DNA binding and dimerization domain, and of a C-terminal trans-activation domain necessary for the integration of the upstream signals (6). SRF contains several phosphorylation sites, including serine 103, known to affect the interaction of SRF with its DNA recognition sequence (7). This serine 103 has been shown to be phosphorylated by pp90rsk, MAPKAP-K2 (MK2), and CaM kinases II and IV (8–10). The TCF is composed of transcription factors of the Ets family, including Elk-1 (11). TCF binds to a purine-rich sequence (CAGGAT) adjacent to the SRE on the c-fos promoter and associates with the SRF (12, 13). The transcriptional activity of TCF proteins is induced after phosphorylation by the mitogen-activated protein kinase (MAPK) family activated by mitogenic and stress signals (6). However, depending on the cell type, some mitogens activate a TCF-independent pathway targeting SRF via the phosphatidylinositol 3-kinase (PI-3K) (14), or via the Rho family of GTPases (15).

The Vav family of guanine nucleotide exchange factors (GEFs) represent a critical link between antigen receptor-coupled protein-tyrosine kinases and the signaling pathways controlled by the Rho family of GTPases (16–18). Vav GEFs are highly homologous proteins composed of a catalytic Db1 homologous (DH) domain, a pleckstrin homology (PH) domain, one Src homology 2 (SH2) domain, and two SH3 domains. Although Vav1 is mostly restricted to hematopoietic cells (19), Vav2 and Vav3 display a much broader tissue expression (20–22). Vav proteins are tyrosine-phosphorylated through the stimulation of diverse receptors, including the epidermal and the platelet-

† This work was supported in part by INSERM, the Fondation pour la Recherche Médicale, and the Association pour la Recherche sur le Cancer. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Recipient of a doctoral fellowship from the Ministère de l’Enseignement Supérieur et de la Recherche.

‡ To whom correspondence should be addressed: INSERM U343, IFR50, Hôpital de l’Archet, 06202 Nice Cedex 3, France. Tel.: 33-4-92-15-77-00; Fax: 33-4-92-15-77-09; E-mail: deckert@unice.fr.

** To whom correspondence should be addressed: INSERM U343, IFR50, Hôpital de l’Archet, 06202 Nice Cedex 3, France. Tel.: 33-4-92-15-77-09; E-mail: deckert@unice.fr.

1 The abbreviations used are: SRE, serum response element; AP-1, activating protein-1; BCR, B cell receptor; ERK, extracellular signal-regulated kinase; GEF, guanine nucleotide exchange factor; IL-2, interleukin-2; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; SH, Src homology; PH, pleckstrin homology; DH, Dbl homologous; SRF, serum response factor; TCF, ternary complex factor; TCR, T cell receptor; TAg, T antigen; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; EMSA, electrophoretic mobility shift assay; PI-3K, phosphatidylinositol 3-kinase; CA, constitutively active form; mAb, monoclonal antibody; HA, hemagglutinin; MBP, myelin basic protein; CaM, calmodulin.
derivation of growth factor receptors, integrins, and B and T cell antigen receptors (BCRs and TCRs) (17). The recruitment of Vav1 into large molecular scaffolds regulates several cell processes, such as activation of the MAPK pathway and gene activation. For example, Vav1 was involved in NFAT (23, 24), NF-κB (25), and AP-1 activation (26). One effector of Vav proteins could be the Ste-20-related p21-associated kinase 1 (Pak1), which is activated by GTP-bound Rac1 or Cdc42 (27). In lymphocytes, Pak1 activation is involved in TCR-mediated actin polymerization (28), and in the activation of p38MAPK (29), and NFAT (30). Moreover, a trimolecular complex composed of the adapters SLP-76 and Nck, and Vav1, has been implicated in the activation of Pak1 following TCR stimulation (28). Thus, Pak1 could be an important element of the signaling pathways controlled by Vav proteins.

Vav proteins appear to act as central components, which can integrate extracellular signals to activate multiple pathways leading to gene transcription in lymphocytes. Recently, we showed that Vav1 and Vav2 activates c-fos SRE upon TCR stimulation (31). However, how Vav proteins regulate SRE-dependent transcription in lymphocytes remains largely unknown. In this study, we show that Vav1 couples TCR stimulation to SRE-dependent transcription via a MEK-dependent pathway. TCR stimulation leads to the phosphorylation of SRF on serine 103 and increases binding of SRF complexes to SRE. Overexpression of Vav1, which partially mimics TCR stimulation, promotes c-fos SRE transcriptional activity through SRF activation. SRF activation can occur through a signaling cascade consisting of Rac1/Cdc42-Pak1-MEK, but independently of the PI-3K pathway. The physiological relevance of our findings is supported by the inhibition of the IL-2 gene transcriptional activity by a dominant negative SRF mutant. Interestingly, Vav2 also enhances SRF activity via the Rho GTPases family. This report establishes the Vav family proteins as a direct link between TCR and SRF-dependent transcription.

**Experimental Procedures**

**Antibodies and Reagents—**Anti-CD3 (OKT3) and anti-Myc (9E10) monoclonal antibodies (mAbs) were purified from hybridoma supernatants. Anti-HA mAb (12CA5) was from Boche (Meylan, France). Anti-phospho-MEK, anti-phospho-ERK1/2, and anti-phospho-p38 antibodies were from Cell Signaling Technology (Beverly, MA). Antibodies against p38, SRF, Akt, and Pak1 were provided by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-ERK1/2 antibody was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Anti-phosphoserine 103-\(\text{p}^{\text{Thr}}\) was kindly provided by M. Greenberg. Anti-N-terminal SRF for electrophoretic shift mobility assay (EMSA) and anti-MEK antibodies were kindly provided by J. C. Chambard. Anti-phosphoserine 473-\(\text{p}^{\text{Thr}}\) antibody was from New England Biolabs (Beverly, MA). Culture media and oligonucleotides were from Invitrogen (Groningen, Netherlands). Myelin basic protein (MBP) was from Sigma. U0126, SB203580, and Ly294002 were obtained from Promega (Madison, WI) and Calbiochem (Darmstadt, Germany), respectively.

**Plasmids—**The constructs encoding Myc-tagged Vav1 (24), Myc-tagged Vav1 mutants (\(\text{AP}^2\), L213A, R695L (26), and Myc-tagged Vav2 (31) have been described before. Dominant negative Rac1 (Rac1N17), Cdc42 (Cdc42N17), RhoA (RhoA197), and Pak1 (PakKR) were a gift of M. Schwartz and J. Chernoff, respectively. Constitutive active MEK1 (MEK118), RhoA (RhoAN19), and Pak1 (PakKR) were obtained from C. M. Schwartz and J. Chernoff, respectively. Constitutive active MEK1 (MEK118), RhoA (RhoAN19), and Pak1 (PakKR) were a gift of J. Chernoff. Constitutive active MEK1 (MEK118), RhoA (RhoAN19), and Pak1 (PakKR) were obtained from C. M. Schwartz and J. Chernoff, respectively.

**Immunoprecipitations and Immunoblotting—**Cells were left unstimulated or stimulated for 5 min with anti-CD3 mAb (5 µg/mL), anti-CD28 mAb (5 µg/mL), or anti-CD28 mAb in ice-cold lysis buffer (1% Triton X-100 in 150 mM NaCl, 50 mM HEPES, pH 7.4, 5 mM NaF, 5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 10 µg/mL aprotinin, 10 µg/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride) for 15 min on ice. Lynasts were clariﬁed by centrifugation at 15,000 × g for 10 min at 4°C, and protein concentra- tion was determined using bicinchoninic acid and protein assay (Pierce). Cleared lysates were directly resolved by SDS-PAGE and analyzed by immunoblotting or incubated for 3 h at 4°C with the indicated antibodies and protein G-Sepharose beads (Sigma). Pellets were then washed three times with ice-cold lysis buffer containing 0.2% Triton X-100 and resuspended in SDS sample buffer. Eluted samples from immunoprecipitations or whole cell lysates were separated by SDS-PAGE and analyzed by immunoblotting. Reactive proteins were visualized by enhanced chemiluminescence (ECL).

**Pak1 Kinase Assays—**Pak1 was immunoprecipitated from lysates of 10 × 10⁶ cells using anti-Pak1 antibodies bound to protein G beads. The immune complexes were washed twice with ice-cold lysis buffer and two times with kinase buffer containing 50 mM Tris, pH 7.5, 100 mM NaCl, 10 mM MgCl₂. Beads were resuspended in 40 µL of kinase buffer containing 5 µg of MBP (Sigma), and reactions were initiated by the addition of 10 µL of kinase buffer containing 5 µL ATP and 10 µCi of [γ-32P]ATP. Reactions proceeded 20 min at 30°C and were stopped by addition of sample buffer for electrophoresis. Reactive proteins were separated on SDS-PAGE. Gels containing radioactive proteins were dried and exposed for 4 h at –80°C.

**Reporter Assays—**For luciferase assays, transfected Jurkat cells (5 × 10⁶ cells) were left unstimulated or stimulated with anti-CD3 mAb as described in the legend to each figure. Cells were washed twice in phosphate-buffered saline and lysed in 100 µL of reporter lysis buffer. Luciferase was assayed using the Promega luciferase assay system and a luminometer (Lumat, EG&G Berthold). Luciferase activity was determined using three- to eight-fold increased as compared to the basal activity seen in unstimulated mock-transfected cells. For monitoring Elk-1 activation, Jurkat cells were co-transfected with GAL4BD: Elk-1 ×5-GAL4-lucifere in the presence or not of indicated expression plasmids. pEFβ-galactosidase was included as an internal transfection control to normalize luciferase reporter activity. For the β-galactosidase assay, 20 µL of the supernatants were incubated at 60°C for 20 min. Samples were lysed in 100 µL of SDS-PAGE loading buffer containing 350 mM NaCl, 20% glycerol, 1% Nonidet P-40, 1 mM MgCl₂, 0.5 mM EDTA, 0.1 mM EGTA. Double-stranded synthetic SRE sequence (5’-GGATGCACATAGGACATCTC-3’) was [γ-32P]ATP-end-labeled using T4 polynucleotide kinase (Amersham Biosciences). Ten µL of cellular proteins were preincubated in a buffer containing 10 mM Tris, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 4% glycerol, 80 µg/mL salmon sperm DNA, 15 µg/mL poly(dI-dC) for 10 min on ice. Then, 30,000–50,000 cpm of [γ-32P] labeled probe were added to the binding reaction for 20 min at room temperature. For competition experiments, a 50-fold excess of unlabeled oligonucleotides was added during preincubation. For supershift assays, 1 µL of anti-SRF or anti-Elk-1 antibodies were added to cellular extracts in the binding reaction buffer and incubated for 10 min on ice. DNA-protein complexes were resolved by electrophoresis on 8% polyacrylamide gels (37.5/1 acrylamide/bisacrylamide) in 0.5x TBE buffer (22.5 mM Tris borate, 0.5 mM EDTA, pH 8) for 3 h at 150 V. Gels containing radioactive SRE probe complexes with proteins were then dried and exposed for 4 h at –80°C.

**RESULTS**

**TCR Engagement Activates SRF—**SRF can be activated by several mitogenic stimuli and plays a critical role in the transcription of the SRE-regulated gene c-fos, a critical component of the AP-1 complex. Although SRF is widely expressed in hematopoietic cells (35), its activation by lymphocyte antigen receptors has not been studied so far. To understand the reg-
Nuclear extracts from Jurkat cells formed two complexes with 1 fos increases SRF binding to c-SRE.

The results shown are representative of three separate experiments.

TCR Engagement Activates SRF via a MEK-dependent Pathway—The MAPK pathway regulates SRE-dependent transcription by phosphorylating Elk-1 but also SRF via the activation of upstream kinases. To examine the role of MAPK pathway on SRE activation by TCR, Jurkat cells transfected with SRF reporter construct were incubated with different amounts of the MEK inhibitor U0126 and the p38 inhibitor SB203580, and stimulated with an anti-CD3 antibody. Although U0126 rapidly decreased SRF activation by TCR, SB203580 slowly decreased it (Fig. 2A). Similarly, TCR-induced IL-2 promoter activity was blocked by SRF mutant (Fig. 2C). As a control, SRF mutant did not affect TCR-induced Elk-1 transactivation (Fig. 3B). Overexpression of SRF mutant was assayed by immunoblotting with an anti-HA antibody (Fig. 2, B–D, insets). Taken together, these results show that SRF activation by TCR is required to promote c-fos SRE activation and IL-2 gene transcriptional regulation in lymphocytes.

TCR engagement induces SRF phosphorylation and increases SRF binding to c-fos SRE. A, Jurkat cells were left unstimulated or stimulated for the indicated times with an anti-CD3 mAb (5 µg/ml) or phorbol 12-myristate 13-acetate (100 ng/ml) plus ionomycin (1 µg/ml) for 5 min. Endogenous SRF was immunoprecipitated from the lysates with an anti-SRF antibody or with a control Ig and subjected to Western blot analysis using an anti-phospho-SRF (upper panel) or anti-SRF (lower panel) as a control of endogenous SRF expression. B, Jurkat cells were left unstimulated or stimulated with anti-CD3 mAb (5 µg/ml) for the indicated times. Nuclear extracts were prepared and analyzed by EMSA, using γ-32P-labeled SRE oligonucleotide probe. Specificity of the complexes was assessed by competition with a 50-fold excess of unlabeled SRE probe. Nonspecific binding is indicated as NS. The results shown are representative of three separate experiments. C, Jurkat cells were left unstimulated (−) or stimulated (+) with an anti-CD3 mAb (5 µg/ml) for 5 min. Nuclear extracts were incubated with 1 µl of anti-SRF or anti-Elk-1 antibodies and analyzed by EMSA, using γ-32P-labeled SRE oligonucleotide probe.
tion-PCR analysis (data not shown). These results show that both MEK/ERK and p38 pathways are required for SRF-dependent transcription induced following TCR engagement.

SRF Activation by TCR Requires Vav1

— Vav1 activates the MEK/ERK pathway in T cells (25, 36), but its role in early gene activation is unclear. Therefore, we examined how Vav1 regulates SRE activity. First, Jurkat cells were transfected with Vav1 along with SRE, SRE\( \Delta \)Ets, or SRE\( \Delta \)SRF reporter constructs followed by anti-CD3 stimulation. As shown in Fig. 4A, overexpression of Vav1 increased both basal and TCR-induced SRE stimulation (6- and 11-fold increase, respectively). These increases were weaker in the absence of the Ets binding site but were still notable (4- and 6-fold increase, respectively). Consistent with Fig. 2A, SRE\( \Delta \)SRF activation by Vav1 was abrogated. Vav1 overexpression was confirmed by immunoblot analysis (Fig. 4A, inset). Jurkat cells overexpressing dominant negative Vav1 mutants (DH-mutated (L213A), or SH2-mutated (R695L)) exhibited a strong reduction of SRF activity, whereas PH-deleted (\( \Delta \)PH) Vav1 had no significant effect (Fig. 4B). As controls, TCR-induced SRE, c-fos promoter, and IL-2 promoter

---

**FIG. 2.** SRF is required for TCR-induced SRE-dependent transcription. A, Jurkat-TAg cells were transfected with SRE or SRE mutants luciferase reporter plasmid (10 \( \mu \)g each) and cultured for 24 h. Cells were left unstimulated (open bars) or stimulated (dark bars) with anti-CD3 mAb (5 \( \mu \)g/ml) for the final 6 h and lysed for luciferase assay. Bars represent the mean ± S.D. of triplicate samples. The data shown are representative of three independent experiments. Jurkat-TAg cells were transfected with empty vector, and HA-tagged SRF mutant (SRF 1–338, 10 \( \mu \)g) along with SRF luciferase reporter (10 \( \mu \)g) (B) or IL-2 promoter (10 \( \mu \)g) (C), and stimulated and analyzed as in panel A. D, Jurkat-TAg cells were transfected with empty vector, and HA-tagged SRF mutant (SRF 1–338, 10 \( \mu \)g) along with Gal4BD:Elk-1 (2 \( \mu \)g) and 5×GAL4-luciferase (5 \( \mu \)g), cultured for 36 h. Cells were left unstimulated (open bars) or stimulated (dark bars) with anti-CD3 mAb (5 \( \mu \)g/ml) for the final 12 h and lysed for luciferase assay. Lysates were analyzed for the expression of SRF mutant by anti-HA immunoblotting.

**FIG. 3.** TCR-induced SRF activation depends on MEK activity. A, upper panel, Jurkat-TAg cells transfected with SRF luciferase reporter were cultured for 24 h and preincubated for 2 h with indicated concentrations of U0126 or SB203580. Cells were then left unstimulated or stimulated with an anti-CD3 mAb for the final 6 h of culture and lysed for luciferase assay. Lower panel, Jurkat cells were preincubated 2 h with Me\( \_\_ \)SO (DMSO), U0126 (20 \( \mu \)M), or SB203580 (20 \( \mu \)M) and left unstimulated (−) or stimulated (+) with an anti-CD3 mAb for 5 min. Lysates were immunoblotted with an anti-phosphoERK1/2 and anti-ERK1/2 or anti-phospho-p38 and anti-p38 antibodies. B, Jurkat cells, preincubated with Me\( \_\_ \)SO, U0126 (20 \( \mu \)M), or SB203580 (20 \( \mu \)M) for 2 h, were left unstimulated (−) or stimulated (+) with anti-CD3 mAb (5 \( \mu \)g/ml) for 5 min. Upper panels, endogenous SRF was immunoprecipitated from the lysates with an anti-SRF antibody or with a control Ig and subjected to Western blot analysis using an anti-phosphoSRF or anti-SRF as a control of endogenous SRF expression. Lower panel, Jurkat cells were treated as previously described. Nuclear extracts were analyzed by EMSA, using \( \gamma \)-\( \beta \)-labeled SRE oligonucleotide probe. C, Jurkat-TAg cells transfected with c-fos promoter luciferase reporter or SRE luciferase reporter were cultured for 36 or 24 h, respectively, and preincubated with indicated concentrations of U0126 for 2 h. Cells were left unstimulated (−) or stimulated (+) with an anti-CD3 mAb for the final 12 or 6 h of culture, respectively, and lysed for luciferase assay.
activations were also inhibited by dominant negative Vav1 mutants (Fig. 4, C, D, and E, respectively). Proper expression of each transfected protein was confirmed by immunoblot analysis (Fig. 4, B and E, insets). Second, to assess whether MEK could be involved in SRF activation by Vav1, Jurkat cells transfected with Vav1 and the SRF reporter were incubated with indicated concentrations of U0126 (Fig. 5A). U0126 inhibited SRF activation by Vav1 up to 70%. To confirm the role of MEK in SRF activation by TCR, Jurkat cells were transfected with a constitutively active form of MEK (CA-MEK) and SRF reporter construct and activated with an anti-CD3 antibody. Fig. 5B shows that CA-MEK strongly increased SRF activation by TCR. An in vitro kinase assay on anti-Pak1 immune complexes isolated from activated Jurkat cells confirms that Pak1 activity was increased following TCR stimulation, as visualized by phosphorylation of the exogenous substrate MBP. As a control of Pak1 expression, immunoblot analysis was performed on immune complexes (Fig. 5C). This result shows that TCR engagement activates SRF by a Vav1-Rac1/Cdc42-Pak1 pathway.
TCR Ligation Activates SRF via a PI-3K-independent Pathway—The PI-3K pathway mediates SRF-dependent transcription in some cellular types (14, 38). To investigate whether this pathway is implicated in SRF activation upon TCR engagement, we transfected Jurkat cells with Vav1 and the SRF reporter. Cells were then preincubated or not with Ly294002, an inhibitor of the PI-3K. Fig. 7A shows that Ly294002 affected neither TCR- nor Vav1-induced SRF activation. To test the efficacy of the inhibitor, we measured the phosphorylation of Akt, a downstream effector of the PI-3K pathway, using an antiserum specific for Akt phosphoserine 473. Fig. 7B shows that Ly294002 inhibited Akt phosphorylation in a dose-dependent manner. Next, Jurkat cells were transfected with constitutive active forms of the catalytic subunit of the PI-3K (p110*) along with either SRF reporter or NF-κB reporter, as a control. As shown in Fig. 7C, p110* had no significant effect on TCR-induced SRF activation, whereas it increased TCR-induced NF-κB activation (Fig. 7D), consistent with previous studies (39). Taken together, these results indicate that TCR and Vav1 may activate SRF independently of the PI-3K pathway.

Vav Proteins Activate SRF via a RhoA-dependent Pathway—We recently showed that Vav2 can promote SRE activation through a Rac1/Cdc42-Pak1-MEK-dependent pathway. A, Jurkat-TAg cells were transfected with empty vector or a combination of Myc-tagged Vav1 and dominant negative Rac1 (Rac1N17), Cdc42 (Cdc42N17), and Pak1 (PakKR) mutants (10 μg each) along with a SRF luciferase reporter (10 μg) and cultured for 24 h. Cells were left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay. B, Jurkat-TAg cells were transfected with Myc-tagged PakKR (30 μg each) and the corresponding empty vector as a negative control. After 24 h, cells were left unstimulated (−) or stimulated (+) with anti-CD3 mAb (5 μg/ml) for 5 min. Lysates were subjected to immunoblot analysis using antibodies against phospho-MEK, MEK1, Myc, and Vav. Densitometric quantification of MEK phosphorylation is shown (lower panel). C, TCR engagement promotes Pak1 activation. Jurkat cells were left unstimulated (−) or stimulated (+) with an anti-CD3 mAb (5 μg/ml) for 2 min and lysed, and anti-Pak1 immune complexes were assayed for a kinase assay using MBP as an exogenous substrate where indicated. The same immune complexes were analyzed for Pak1 expression by immunoblotting with an anti-Pak1 antibody.
stimulated with an anti-CD3 mAb (5 dark bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay. B, Jurkat cells were incubated with indicated concentrations of Ly294002 for 2 h. Cells were lysed and lysates analyzed by anti-phosphoserine 473-Akt (upper panel) or anti-Akt (lower panel) immunoblotting. C, Jurkat-TAg cells were transfected with empty vector or constitutive forms of PI-3K (p110*), along with the SRF luciferase reporter (10 μg) or NF-κB luciferase reporter (10 μg). D, cells were then left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay.

In vitro studies have shown that Vav2 can act as a potent GEF for Rac1 and Cdc42, but also for RhoA (40). Moreover, SRF exhibits a strong dependence on functional RhoA (15). Jurkat cells were transfected with a combination of Myc-tagged Vav1 (5 μg) or Myc-tagged Vav2 (5 μg) and RhoA N19 (10 μg) and their respective corresponding empty vectors, along with a SRF luciferase reporter (10 μg) (A) or NFAT luciferase reporter (10 μg) (B), and cultured for 24 h. Cells were left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay. Lysates were analyzed for the expression of Vav1 and Vav2 by immunoblotting with an anti-Myc mAb (inset). C, Jurkat-TAg cells were transfected with RhoN19 (10 μg) along with Gal4BD:Elk-1 (2 μg) and 5×GAL4-luciferase (5 μg), cultured for 36 h, and left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 12 h of culture, and lysed for luciferase assay.

FIG. 7. TCR engagement activates SRF via a PI-3K-independent pathway. A, Jurkat-TAg cells transfected with empty vector or Myc-tagged Vav1 (5 μg) along with a SRF luciferase reporter (10 μg) were cultured for 24 h and preincubated with Ly294002 (5 μM) for 2 h or ethanol as a negative control. Cells were left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay. B, Jurkat cells were incubated with indicated concentrations of Ly294002 for 2 h. Cells were lysed and lysates analyzed by anti-phosphoserine 473-Akt (upper panel) or anti-Akt (lower panel) immunoblotting. C, Jurkat-TAg cells were transfected with empty vector or constitutive forms of PI-3K (p110*) along with the SRF luciferase reporter (10 μg) or NF-κB luciferase reporter (10 μg). D, cells were then left unstimulated (open bars) or stimulated (dark bars) with an anti-CD3 mAb (5 μg/ml) for the final 6 h of culture and lysed for luciferase assay.

Vav proteins play an essential role in the activation of multiple transcription factors, which in turn control gene expression leading to T cell activation and proliferation. SRE-regulated early genes, such as c-fos, participate in the formation of AP-1 complexes (3), but the biochemical pathways leading to early gene activation in lymphocytes remain uncharacterized. Vav1 regulates AP-1 activation in T cells following JNK activation and c-Jun phosphorylation (26). The activity of AP-1 complex can be regulated by a second mechanism that requires de novo transcription of c-fos. We recently showed that Vav proteins activate c-fos SRE in T cells (31). However, the mechanisms by which Vav1 can regulate c-fos SRE transcriptional activity following TCR engagement remain unclear.

Here we show that TCR stimulation regulates SRF-dependent transcription through a pathway connecting Vav proteins to Rho family GTPases and the serine/threonine kinases Pak1 and MEK, but independently of the PI-3K. Our findings provide the first evidence of a signaling cascade activating SRF-dependent transcription by Vav proteins, which could participate in the regulation of lymphocyte proliferation (Fig. 9). We
clearly demonstrate that TCR engagement increases c-fos SRE and SRF activation in Jurkat cells. Surprisingly, we observed that serum has no effect on SRE activation in Jurkat cells and does not interfere with TCR-increased SRE activation (data not shown). One explanation could be that Jurkat T cells are leukemic cells, which are relatively serum-independent. However, our data also indicate that SRF activation by TCR is a critical step for early gene activation in T cells. TCR activation is often assisted by costimulatory molecules present on the T cell surface such as CD28. This costimulation leads to stronger and more sustained phosphorylation and activation of Vav1 than when each receptor is cross-linked alone, indicating that Vav1 can integrate extracellular signals from multiple membrane receptors. Previous studies have shown that CD3/CD28 costimulation is required for AP-1 activation, including at the level of c-fos (41). On the other hand, CD28 costimulation has been shown to differentially regulate c-fos and c-jun expression (42). Although Vav1 overexpression, which partially mimics TCR and CD28 costimulation, increased SRF activation, we did not observe a costimulatory effect of CD28 and CD3 on either SRF or SRE activation (data not shown). Recent studies have shown that CD28 preferentially activates a PI-3K-dependent pathway (43, 44). In our cellular system, SRF activation by TCR occurs via a PI-3K-independent pathway, which could explain the lack of effect of CD28 costimulation. We also observed that deletion of the SRF binding sites on SRE abrogated Vav1-induced SRE activation. By contrast, deletion of the TCF binding sites on SRE had only limited effect on SRE activation by TCR or Vav1. This strongly supports the notion that SRF binding sites on SRE had only limited effect on SRE activation. Vav1-induced SRE activation. By contrast, deletion of the TCF could potentiate SRE-dependent transcription in lymphocytes and chromatin remodeling events induced by acetylation of specific histones (45, 46).

SRE-regulated early genes control long term gene expression, cell growth, and survival. The physiological relevance of our findings is highlighted by the fact that the transcriptional activity of the IL-2 gene, a critical cytokine regulating T cell proliferation, is blocked by a dominant negative SRF mutant, following TCR stimulation and/or Vav1 overexpression (data not shown). Consistently, we showed that Vav1-induced AP-1 activation plays a major role in IL-2 transcriptional regulation in T cells (26). Moreover, T cells from Vav1−/− mice fail to proliferate in response to TCR stimulation, because they essentially fail to secrete IL-2 (25). Interestingly, the loss of Vav2 also results in impaired BCR-dependent proliferation, suggesting that a similar process may regulate B cell growth (47, 48). These observations fully support our findings that the Vav family play an important role in the regulation of lymphocyte SRE-dependent early gene expression, cytokines synthesis, and cell proliferation.

Previous studies have shown that SRE-regulated early gene activation occurs via a MAPK-dependent pathway. Supporting a role of MEK in the regulation of early gene transcription, the MEK inhibitor PD98059 inhibits c-fos induction (49, 50). Vav1-deficient T cells showed severe defects in ERK1/2 activation (25). Consistently, we show that the MEK inhibitor U0126 blocks both TCR- and Vav1-enhanced SRF activation. Although activation of Elk-1 by ERK1/2 is well described, the activation of SRF by phosphorylation is less clear. SRF can be phosphorylated by pp90rsk, a known effector of ERKs, CaM kinases II and IV, and the MAPKAP-2 (MK2), which is regulated by p38 MAPK (10). SRF phosphorylation could facilitate access of SRF to SRE (7). We clearly showed that TCR stimulation resulted in the phosphorylation of SRF on its serine 103 and modulated the binding of SRF complexes to SRE in a MEK- and p38-dependent pathway. Considering the critical role of Vav1 in ERK1/2 activation in T cells (25, 36), pp90rsk could be a good candidate for connecting MEK to SRF in T cells. Another possible intermediate is p38 MAPK because Vav1 has been shown to regulate p38 MAPK activity in T cells (51). Supporting this idea, we observed that the p38 MAPK inhibitor SB203580 decreased TCR-induced SRF activation. Thus, identification of the SRF kinases implicated in TCR signaling could be of great interest to understand the regulation of early genes in lymphocytes.

A major question is what are the effectors between Vav1 and MEK connecting the TCR to SRF-dependent pathway? Lim and co-workers (52) have shown that active Rac1 and Cdc42 GTPases are required for Pak1 activation. We show that dominant negative forms of Rac1, Cdc42, and Pak1 blocked SRF activation by TCR and Vav1, indicating that these proteins seem to function downstream of Vav1. The point mutation replacing the lysine 299 in an arginine does not affect the binding of Pak1 CRIB domain to activated Rac1 and Cdc42, suggesting that Pak1 activity per se is required. However, although we observed that TCR engagement stimulates Pak1 activation, we found that overexpression of wild type Pak1 did not induce SRF activation (data not shown). This suggests that Pak1 is necessary but not sufficient to promote SRE activation in T cells. These results are consistent with the findings that overexpression of Pak1 was not able to activate NFAT in T cells (30). The association of Pak1 with Vav1, Nck, and SLIP-76 is required for its activation following TCR cross-linking (28). Consistently, our Vav1 mutants lacking DH (L213A) and SH2 (R695L) functions blocked TCR-induced SRF and c-fos SRE activation (data not shown). The PakKR mutant used in this study conserves its interactive motifs. This rules out the possibility that its dominant negative effect on SRF activation is a consequence of a defect in forming molecular complexes. Studies in fibroblasts have shown that Pak1 phosphorylated MEK at serine 298, a site important for MEK-Raf1 interaction (37). We show that PakKR decreased MEK activation following TCR engagement, indicating that Pak1 similarly acts upstream of MEK in the pathway connecting the TCR to SRF activation.
Interestingly, Pak1 did not totally block MEK activation by TCR, suggesting that Pak1 and/or MEK might integrate signals from other pathways known to regulate SRE, such as the Ras pathway.

Recent findings have shown that SRF activation can occur through either RhoA-dependent or RhoA-independent pathways (53). PI-3K has been implicated in the signaling to SRF depending on extracellular stimuli (14, 54). PI-3K products from other pathways known to regulate SRE, such as the Ras pathway.
Vav1 Couples T Cell Receptor to Serum Response Factor-dependent Transcription via a MEK-dependent Pathway
Céline Charvet, Patrick Aubergé, Sophie Tartare-Deckert, Alain Bernard and Marcel Deckert

J. Biol. Chem. 2002, 277:15376-15384.
doi: 10.1074/jbc.M111627200 originally published online February 21, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111627200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 59 references, 32 of which can be accessed free at http://www.jbc.org/content/277/18/15376.full.html#ref-list-1