TCR-mediated Erk activation does not depend on Sos and Grb2 in peripheral human T cells

Nicole Warnecke^{1*}, Mateusz Poltorak^{1*}, Bhavani S. Kowtharapu¹, Boerge Arndt^{1†}, James C. Stone², Burkhardt Schraven^{1,3} & Luca Simeoni¹⁺

¹Institute of Molecular and Clinical Immunology, Otto-von-Guericke University, Magdeburg, Germany, ²Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada and ³Department of Immune Control, Helmholtz Centre for Infection Research, Braunschweig, Germany

Sos proteins are ubiquitously expressed activators of Ras. Lymphoid cells also express RasGRP1, another Ras activator. Sos and RasGRP1 are thought to cooperatively control full Ras activation upon T-cell receptor triggering. Using RNA interference, we evaluated whether this mechanism operates in primary human T cells. We found that T-cell antigen receptor (TCR)-mediated Erk activation requires RasGRP1, but not Grb2/Sos. Conversely, Grb2/Sos—but not RasGRP1—are required for IL2-mediated Erk activation. Thus, RasGRP1 and Grb2/Sos are insulators of signals that lead to Ras activation induced by different stimuli, rather than cooperating downstream of the TCR.

Keywords: T cells; Sos; Grb2; RasGRP1; Erk

EMBO reports (2012) 13, 386–391. doi:10.1038/embor.2012.17

INTRODUCTION

Signalling via the Erk cascade is essential for cell-fate decisions in many cell types including T lymphocytes [1]. On ligation of the T-cell antigen receptor (TCR), the activation of the Erk cascade is thought to occur through the action of two distinct GEFs, RasGRP1 and the ubiquitously expressed Sos proteins [2]. The activation of both RasGRP1 and Sos depends on the transmembrane adaptor linker for activation of T cells (LAT), which might therefore activate Ras via two distinct pathways: (i) PLC γ 1-DAG-RasGRP1 and (ii) Grb2-Sos.

A key finding on Ras-Erk regulation in T cells came from recent studies based on lymphoid cell lines, thymocytes and *in silico* simulations showing that Ras activation is further controlled by an

unusual interplay between RasGRP1 and Sos [3,4]. This model has important implications for T-cell function, as it helps to explain how antigenic peptide/major histocompatibility complexes, which are usually rare during *in vivo* infections, induce productive T-cell activation [4]. These studies propose that, on initial receptor triggering, Ras is exclusively activated by RasGRP1. Successively, RasGTP will prime Sos thereby activating a positive feedback loop and allowing full T-cell activation [4].

Thus, the current model postulates that the coordinated action of RasGRP1 and Sos regulates the magnitude of Ras-Erk activation. However, it has not yet been demonstrated whether this model for TCR-mediated Ras-Erk is also valid in primary human T cells. Given the central role of Erk activation in cell-fate specification processes induced by TCR triggering, we have examined the molecular events leading to Erk activation in peripheral human T cells in more detail.

Surprisingly, we found that Sos proteins and Grb2 are not limiting for full Erk activation upon TCR stimulation in primary human T cells. Thus, this study shows that the contribution of Sos to the regulation of the magnitude of Erk activation in primary human T cells is considerably different from that predicted by the current model.

RESULTS

Sos and Grb2 are not limiting for Erk activation

On the basis of overexpression experiments in Jurkat T cells and computer simulations, it has been postulated that Sos is important in inducing robust Ras signalling in T cells [3,4]. To evaluate whether Sos also has a similar role in primary T cells, we performed short interfering RNA (siRNA)-mediated downregulation of Sos. We initially evaluated the role of Sos1. As shown in Fig 1A, transfection with RNA duplex efficiently suppressed Sos1 expression in peripheral human T cells. Next, human T cells were treated with two different CD3 ϵ monoclonal antibodies, MEM92 or OKT3. As expected, suppression of Sos1 did not alter the activation status of the upstream signalling molecules LAT, PLC γ 1 and ZAP-70 (data not shown). However, when we analysed Erk activation we surprisingly found that Erk phosphorylation was not reduced upon TCR stimulation in primary T lymphocytes (Fig 1A). In the case of stimulation with MEM92, Erk phosphorylation was even slightly enhanced (Fig 1A).

¹Institute of Molecular and Clinical Immunology, Otto-von-Guericke University, Leipziger Strasse 44, Magdeburg 39120, Germany

²Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

³Department of Immune Control, Helmholtz Centre for Infection Research, Inhoffenstrasse 7, Braunschweig 38124, Germany

*These authors contributed equally to this work

[†]Present address: Diabetes Centre, Department of Medicine-Innenstadt, Ludwig-Maximilian University, Munich 80336, Germany

⁺Corresponding author. Tel: +49 391 6717894; Fax: +49 391 6715852; E-mail: luca.simeoni@med.ovgu.de

Fig 1 | TCR-mediated activation of Erk does not depend on Sos in primary T cells. Peripheral human T cells were transfected with Ctrl, Sos1-specific (A) or Sos1/Sos2 (B) siRNA and cultured for 72 h. Cells were stimulated with CD3 mAbs (clone OKT3 or MEM92) for the indicated times. Cell lysates were analysed by immunoblotting using the indicated Abs. Immunoblots verifying Sos1 and Sos2 downregulation are shown. Bands in A were quantified using the ImageQuant software and values were normalized to the corresponding β-actin signal. Graph in A show the phosphorylation levels of Erk1/2 as arbitrary units $\pm$ s.e.m. of six experiments. One representative experiment out of four is shown in B. Numbers below the band indicate expression levels or fold induction of phosphorylation compared with Ctrl. Ab, antibody; Ctrl, control; mAb, monoclonal antibody; siRNA, short interfering RNA; TCR, T-cell antigen receptor.

To check whether the highly homologous Sos2 might compensate for Sos1, we suppressed both Sos1 and Sos2 expression. As shown in Fig 1B, downregulation of both Sos proteins also did not affect but rather slightly enhanced Erk activation. Similar results were obtained by analysing the level of phosphorylated Erk by flow cytometry (supplementary Fig S1 online). Thus, these data indicate that, in contrast to the proposed model, Sos proteins are not limiting for full Ras-Erk activation in primary human T lymphocytes.

Sos constitutively binds the adaptor protein Grb2 by its two SH3 domains [5]. It is well established that, during T-cell activation, this constitutive interaction recruits Sos to the plasma membrane upon binding of the Grb2-SH2 domain to phosphorylated LAT [6]. The interaction between Sos and Grb2 is also required to activate the Ras-Erk cascade by binding of the Grb2-SH2 domain to phosphorylated Shc, an adaptor protein that directly binds to phosphorylated TCR- ζ immunoreceptor tyrosine-based activation motifs [7]. In addition to Sos, Grb2 could regulate Erk activation via Themis [8]. Hence, given the crucial role of Grb2 in regulating the activation of Ras, we evaluated whether suppression of Grb2 expression in primary human T cells would result in defective Erk activation. The results presented in Fig 2 show that, similarly to Sos, the downregulation of Grb2 did not reduce Erk activation. Rather, Erk activation was slightly enhanced on strong TCR stimulation. Similar results were obtained by using another set of siRNA duplex targeting another region of Grb2 mRNA (data not shown).

To evaluate whether residual expression of Grb2 and Sos might still be sufficient to activate Erk, we simultaneously downregulated Sos1, Sos2 and Grb2. Also under this condition we did not

observe a reduction in Erk activation (supplementary Fig S2 online). In addition, we tested the hypothesis that Grb2 and Sos might have a role in Ras activation in different T-cell subsets. To assess this possibility, the expression of Grb2 and Sos1/Sos2 was suppressed in CD4⁺ naive (supplementary Fig S3 online) and memory T cells (supplementary Fig S4 online). Also in these cells the downregulation of Grb2 and Sos1/Sos2 did not reduce Erk activation.

As our data showed that Sos1 and Grb2 are not crucial for the activation of Erk in primary human T cells, we next analysed whether they might have a role in the activation of other mitogen-activated protein kinases (MAPK). These investigations were motivated by the observation that Grb2^{-/-} thymocytes [9] and thymocytes with Grb2 haploid insufficiency clearly showed weakened TCR-mediated JNK and p38, but normal Erk activation [10]. Therefore, Grb2 and Sos1 might be required for JNK or p38 activation rather than for Erk upon TCR stimulation. However, we found that both molecules also do not influence the activation of JNK and p38 in primary human T cells (supplementary Fig S5 online).

According to the proposed model, Sos1 might function as an amplifier of the Ras-Erk cascade in Jurkat T cells. Therefore, to further test the validity of our approach, we suppressed the expression of Sos1 and Grb2 in Jurkat T cells in an attempt to recapitulate the current model. In marked contrast to primary T cells, we found that downregulation of Sos1 (supplementary Fig S6A online) or Grb2 (supplementary Fig S6B online) resulted in a reduction of Erk activation on both strong and weak TCR

Fig 2 | Knockdown of Grb2 does not reduce Erk phosphorylation in primary T cells. Peripheral human T cells transfected with siRNA duplex for Grb2 or Ctrl were stimulated with CD3 mAb (clone OKT3 or MEM92) for the indicated times. Total cell lysates were prepared and analysed as in Fig 1. Graphs show the phosphorylation levels of Erk1/2 as arbitrary units $\pm$ s.e.m. of at least three independent experiments. Ctrl, control; mAb, monoclonal antibody; siRNA, short interfering RNA.

stimulation in Jurkat T cells, thus confirmed that the prediction made by the model are valid in the Jurkat T cell line.

Altogether, our data demonstrate that, conversely to Jurkat T cells, Sos and Grb2 do not significantly contribute to full TCR-mediated Ras-Erk activation in primary human T cells. Surprisingly, both Sos and Grb2 seem to have a modest inhibitory effect on Erk phosphorylation.

RasGRP1 regulates TCR-mediated Erk activation

To assess how Erk is activated on TCR triggering in primary human T cells, we next investigated the role of another important Ras activator, RasGRP1. To this end, we suppressed RasGRP1 expression by RNA-mediated interference. The data shown in Fig 3A indicate that knockdown of RasGRP1 results in a severe reduction of Erk phosphorylation, but not of another MAPK, p38 upon TCR stimulation (data not shown). The analysis of the intracellular levels of phospho-Erk by flow cytometry confirmed this result (supplementary Fig S1 online). Thus, in addition to be involved in Erk activation in murine thymocytes [11], peripheral CD8⁺ [12,13] and Jurkat T cells [3,14], RasGRP1 is also an important regulator of Ras-Erk activation on TCR stimulation in primary human T lymphocytes.

Interestingly, we found that Erk activation on CD3xCD28 stimulation is less dependent on RasGRP1, as suppression of

RasGRP1 only modestly affected Erk phosphorylation (Supplementary Fig S7 online). This indicates that CD28 costimulation either triggers RasGRP1-independent pathways to activate Erk (for example, by activating a PKC-Raf axis) or that it enhances the activation of the residual RasGRP1.

It has been proposed that, to be activated, Sos requires a priming by active Ras produced by RasGRP1 [3]. Therefore, it is possible that Sos function is masked by the dominant action of RasGRP1 in primary human T cells, and hence, the effect of Sos on Ras-Erk activation cannot be detected when sufficient amount of RasGRP1 are expressed. To address this possibility, we simultaneously suppressed both Sos1 and RasGRP1. If Sos1 and RasGRP1 cooperate to regulate Ras activation, the suppression of both Sos1 and RasGRP1 should affect Erk activation in a more pronounced manner compared with RasGRP1 alone. Surprisingly, we found that the further suppression of Sos1 neutralizes the effect seen with RasGRP1 (Fig 3B). Similar results were obtained when we suppressed both Grb2 and RasGRP1 (supplementary Fig S8 online). Thus, these results suggest that rather than cooperatively, Sos1 and RasGRP1 antagonize one another to regulate Ras activation.

Sos and Grb2 regulate IL-2R-mediated Erk activation

As our data show that Sos and Grb2 are not crucial for TCR-mediated Erk activation in primary human T cells, we next analysed whether they have a role in the activation of Erk downstream of other receptors such as the IL-2R, which is also essential for T-cell activation. Therefore, we generated T-cell blasts in which the expression of Grb2, Sos1/Sos2 and RasGRP1 was suppressed by RNA-mediated interference. Erk activation was analysed on IL-2 stimulation. As shown in Fig 4A,B,D, suppression of Grb2 and Sos1/Sos2 expression significantly reduced IL-2-mediated Erk phosphorylation. Consistent with previously published data [13], we found that the downregulation of RasGRP1 did not affect IL-2-mediated Erk activation (Fig 4C,D). The data in Fig 4 also show that the phosphorylation of STAT3, which does not depend on Grb2/Sos, is not affected.

Collectively, these results show that Grb2/Sos and RasGRP1 are involved in the activation of the Ras-Erk cascade downstream of different receptors. Whereas Grb2/Sos are required for IL-2- but not TCR-mediated Erk activation, RasGRP1 is an activator of Ras downstream of the TCR, but it is not required for IL2 receptor signalling.

Finally, we tested whether suppression of Grb2, Sos1/Sos2 and RasGRP1 affect T-cell activation. In agreement with previously published data [12], we found that suppression of RasGRP1 expression markedly reduced CD3-mediated proliferation (Fig 5). Similarly, also the suppression of Grb2 and Sos1/Sos2 reduced proliferation. However, the effect of Sos1/Sos2 was more pronounced than that of Grb2. It is possible that during proliferation T cells might compensate the loss of Grb2 by other adaptor molecules. In summary, the data indicate that both Grb2/Sos and RasGRP1 are required for T-cell activation, albeit downstream of different receptors.

DISCUSSION

Erk functions as a hub that directs signals coming from different cell-surface receptors (for example, growth factor, chemokine, cytokine and antigen receptors) towards the receptor-specific

Fig 3 | RasGRP1 is required for full Erk activation in primary T cells. Peripheral human T cells transfected with siRNA duplex for RasGRP1 (A) or RasGRP1 and Sos1 (B) and were stimulated as indicated with CD3 mAbs (clone MEM92). Total cell lysates were prepared and analysed as in Fig 1. Graphs show the phosphorylation levels of Erk1/2 as arbitrary units $\pm$ s.e.m. of at least three independent experiments. Statistical significance *** P < 0.001. mAb, monoclonal antibody; siRNA, short interfering RNA.

Fig 4 | Sos and Grb2 regulate IL2-mediated Erk activation. T-cell blasts were generated, transfected with siRNA duplex for Grb2 (A), Sos1/Sos2 (B) or RasGRP1 (C), and stimulated with IL2 as indicated. Total cell lysates were prepared and analysed as in Fig 1. Graphs in D present the mean of the phosphorylation levels as arbitrary units $\pm$ s.e.m. of four independent experiments. Statistical significance *** P < 0.001, * P < 0.05. siRNA, short interfering RNA.

cellular response. How Erk performs its function has now begun to be understood. It seems that signalling through the Erk module is controlled by several upstream regulators that determine the duration, the magnitude and the compartmentalization of Erk activation [15]. Thus, by eliciting different modes of Erk

activation, the generation of a receptor- or ligand-specific cellular outcome is ensured.

By using lymphoid cell lines and *in silico* simulations, it has recently been proposed that RasGRP1 and Sos synergistically function as upstream activators of the Ras-Erk cascade and

Fig 5 | Grb2/Sos and RasGRP1 are required for T-cell activation. Peripheral human T cells were transfected with siRNA duplex for Grb2, Sos1/Sos2, RasGRP1 or siRNA Ctrl. Subsequently, cells were stimulated with plate-bound CD3 for 2 days, pulsed with [³H]-thymidine and processed for standard scintillation counting. Graphs show proliferation expressed as arbitrary units $\pm$ s.e.m. of Grb2-, Sos1/Sos2- and RasGRP1-low T cells compared with Ctrl. Results are from at least three independent experiments. Statistical significance *** $P < 0.001$, * $P < 0.05$. Ctrl, control; siRNA, short interfering RNA.

cooperatively regulate the magnitude of Erk activation on TCR stimulation [3,4,16]. The mechanism described by this model might have important consequences for our understanding of how T cells are selected in the thymus and how they are activated during an immune response. Recently, conditional Grb2^{-/-} [9] and Sos1^{-/-} [17] mice have been generated. The data suggest that both Grb2 and Sos1 are required at different stages during thymocyte development. However, whereas Sos1 contributes to TCR-mediated Erk activation in immature T cells, Grb2 seems to be dispensable.

Here, we have assessed the role of Sos and Grb2 in TCR-mediated Erk activation in mature human T cells isolated from peripheral blood. We found that the Grb2/Sos signalling module does not appear essential for full Erk activation on TCR stimulation in primary human T cells. Our data corroborate recent observations showing that CD4⁺ peripheral T cells from conditional Sos1^{-/-} mice also display normal TCR-mediated Erk activation [17].

In addition, we show that Sos1 and Grb2 are required for Erk activation in Jurkat T cells. Thus, our data demonstrate that the prediction made by the current model of Ras-Erk activation is valid for Jurkat, but not in primary human T cells. The reason for the differential requirement of Grb2/Sos in primary versus Jurkat T cells is not yet clear. Jurkat T cells seem to have the characteristic of immature double-negative thymocytes. It is possible that the requirement of Sos to activate Ras might differ in immature versus mature T cells. This hypothesis seems to be in line with recent observations by Kortum *et al* [17] suggesting that immature T cells require Sos1 to activate Erk, whereas mature T cells are largely Sos1 independent. However, it should also be kept in mind that, although being the best characterized model system to study TCR-mediated signalling, Jurkat are a transformed T-cell line that are known to carry mutations in different genes which could account for the observed differences to primary T cells.

Whereas RasGRP1 is the major activator of Ras in immature CD4⁺CD8⁺ thymocytes [11,12], it seems that mature T cells use both RasGRP1-dependent and -independent pathways to activate Erk. In fact, despite the strong depletion (80%) of RasGRP1 that we

were able to archive in our experiments, Erk phosphorylation was reduced by only about 50%. In line with our findings, recent data show that RasGRP1 deficiency partially affects, but does not completely abrogate TCR-mediated Erk activation, CD69 and CD25 upregulation in peripheral mouse T lymphocytes [12,13].

One of the RasGRP1-independent pathways could be mediated by RasGRF2. However, recent data suggest that loss of RasGRF2 induces no major changes in Erk activation in TCR-stimulated splenic T cells [18]. Thus, it is currently not clear which RasGRP1-independent signalling pathways contribute to Erk activation in primary T lymphocytes.

Our experiments also show that, in addition to being not crucial for TCR-mediated Ras-Erk activation, Sos1 and Grb2 are not limiting for TCR-mediated JNK or p38 activation in primary human T cells. These results were unexpected in light of recent data demonstrating defective TCR-mediated JNK and p38 activation in Grb2^{-/-} [9] and Grb2^{+/-} [10] thymocytes. This once again indicates that thymocytes and peripheral T cells differentially use signalling pathways to activate MAPKs upon TCR stimulation.

Another surprising observation of our study was that down-regulation of Grb2 or Sos slightly enhanced TCR-mediated activation of Erk. This suggests that Grb2 and Sos might function as inhibitory signalling molecules in primary human T cells. In line with this assumption, it has been proposed that Grb2 is required for the assembly of an inhibitory signalling complex in T cells with the lipid phosphatase SHIP1 and the adaptor protein Dok2, which might inhibit Ras upon binding to RasGAP [19]. We have found that the inhibitory effect on TCR-mediated Erk activation was more evident on Grb2 knockdown compared with Sos1 or Sos1/Sos2 suppression. Thus, it is likely that Grb2 performs parts of its inhibitory signalling functions independently of Sos proteins.

An important question arising from our study is whether Sos activity is required for the activation of Ras in mature T cells. We explored the possibility that Sos1 and/or Sos2 regulates Ras activation in signalling pathways mediated by other receptors, such as the IL-2R. This hypothesis is in line with the observation that cytokine receptors can also activate Ras-Erk on recruitment of the Grb2/Sos complex to the phosphorylated receptor by the adaptor protein Shc [20]. We found that Grb2/Sos is indeed required to regulate IL-2-mediated Erk activation, whereas RasGRP1 appears to be dispensable, which is in line with recently published data [13]. Thus, RasGRP1 and Grb2/Sos do not function as integrators, but they might rather function as insulators allowing the cells to distinguish the source of the input. Understanding the spatio-temporal contribution of signals from different receptors such as the TCR, the IL-2R and perhaps also by costimulatory molecules, such as integrins, is essential to predict cellular behaviour in a physiological context [21].

METHODS

Approval for these studies was obtained from the Ethics Committee of the Medical Faculty at the Otto-von-Guericke University, Magdeburg, Germany. Informed consent was obtained in accordance with the Declaration of Helsinki Principles.

Cell culture. Peripheral blood T cells were isolated as previously described [22]. T-cell blasts were generated as previously described [21].

siRNA duplex and transfection. siRNA duplex containing 19 nucleotides with 2 thymidine 3' overhangs were purchased from

Invitrogen. The sequences were as follow: for Sos1: 5'-UUGCCC AUUUAUCAAUUGGTT-3', for Grb2: 5'-CAUGUUUCCCCGCA AUUAUTT-3', for RasGRP1: 5'-GGGUGAGGAGUUAUUAUUG CTT-3', and as negative control we used a Renilla luciferase siRNA duplex 5'-CCAAGUAAUGUAGGAUCAATT-3'. For Sos2 downregulation, a STEALTH pool of three siRNAs (Invitrogen) was used. To achieve efficient downregulation, primary human T cells or T-cell blasts were transfected with siRNA duplex using either the Nucleofection Kit (Amaxa) according to the manufacturer's instruction or the Gene Pulser Xcell (Bio-Rad) as previously described [22]. Cells were collected 72 h after electroporation.

Cell stimulation and western blot analysis. Cell stimulations and cell lysates were prepared as previously described [22]. Anti-CD3 OKT3 (ATCC) and anti-CD3 MEM92 (kindly provided by V. Horejsi, Academy of Sciences of the Czech Republic, Czech Republic) were used for stimulation as hybridoma supernatants. Before experiments, hybridoma supernatants were titrated by two-fold dilution (from 1:1 to 1:64) to determine the appropriate monoclonal antibody concentration resulting in optimal T-cell stimulation (judged by phosphorylated Erk staining). T-cell blasts were stimulated as previously described [21]. Human recombinant IL-2 (Peprotech) was used at 100 U/ml. Western blots were conducted with the following antibodies: anti-phosphospecific antibodies (all from Cell Signaling Technology), anti- β -actin (clone AC-15; Sigma-Aldrich), anti-RasGRP1 [23], anti-Sos1 (C-23), anti-Sos2 and anti-Grb2 (C-23; Santa Cruz Biotechnology). Membranes were probed with the appropriate horseradish peroxidase-conjugated secondary antibodies and developed using the ECL detection system (Amersham Pharmacia).

Proliferation assay. To assess the proliferative capacity, cells were transfected with siRNA duplex as described above, rested overnight and stimulated in 96-well round-bottomed tissue culture plates (Costar; Corning Life Sciences, Acton, MA) coated with CD3 monoclonal antibody (MEM92). Cells were plated at 5×10^4 cells per well in quadruplicates and cultured for 2 days. [3 H]-Thymidine (0.3 μ Ci per well; specific activity, 50 Ci/mmol) was added for the last 8 h, and the plates were collected using a PHD cell harvester (Inotech AG, Basel, Switzerland). Thymidine incorporation was measured by liquid scintillation counting.

Statistics. Statistical analyses were performed using GraphPad Prism (GraphPad Software Inc., San Diego, CA). *P*-values were determined by an unpaired two-tailed Student's *t*-test. Statistical significance is indicated by asterisks.

Supplementary information is available at EMBO reports online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We are grateful to Jonathan Lindquist and Tilo Beyer for critically reading the manuscript and helpful discussion and to Nicole Juelling and Ines Meinert for excellent technical assistance. This work was supported by grants from the German Research Foundation (DFG), FOR-521 [SI861/1], GRK-1167 [TP12] and SFB-854 [TP19].

Author contributions: N.W., M.P. and B.S.K. performed research and analysed data; B.A. and B.S. designed research; J.C.S. contributed vital new reagents; L.S. analysed data, designed research and wrote the paper.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

- Yasuda T, Kurosaki T (2008) Regulation of lymphocyte fate by Ras/ERK signals. *Cell Cycle* **7**: 3634–3640
- Roose J, Weiss A (2000) T cells: getting a GRP on Ras. *Nat Immunol* **1**: 275–276
- Roose JP, Mollenauer M, Ho M, Kurosaki T, Weiss A (2007) Unusual interplay of two types of Ras activators, RasGRP and SOS, establishes sensitive and robust Ras activation in lymphocytes. *Mol Cell Biol* **27**: 2732–2745
- Das J, Ho M, Zikherman J, Govern C, Yang M, Weiss A, Chakraborty AK, Roose JP (2009) Digital signaling and hysteresis characterize ras activation in lymphoid cells. *Cell* **136**: 337–351
- Buday L, Downward J (1993) Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* **73**: 611–620
- Buday L, Egan SE, Rodriguez Viciano P, Cantrell DA, Downward J (1994) A complex of Grb2 adaptor protein, Sos exchange factor, and a 36-kDa membrane-bound tyrosine phosphoprotein is implicated in ras activation in T cells. *J Biol Chem* **269**: 9019–9023
- Zhang L, Lorenz U, Ravichandran KS (2003) Role of Shc in T-cell development and function. *Immunol Rev* **191**: 183–195
- Brockmeyer C et al (2011) T cell receptor (TCR)-induced tyrosine phosphorylation dynamics identifies THEMIS as a new TCR signalosome component. *J Biol Chem* **286**: 7535–7547
- Jang IK, Zhang J, Chiang YJ, Kole HK, Cronshaw DG, Zou Y, Gu H (2010) Grb2 functions at the top of the T-cell antigen receptor-induced tyrosine kinase cascade to control thymic selection. *Proc Natl Acad Sci USA* **107**: 10620–10625
- Gong Q, Cheng AM, Akk AM, Alberola-Ila J, Gong G, Pawson T, Chan AC (2001) Disruption of T cell signaling networks and development by Grb2 haploid insufficiency. *Nat Immunol* **2**: 29–36
- Dower NA, Stang SL, Bottorff DA, Ebinu JO, Dickie P, Ostergaard HL, Stone JC (2000) RasGRP is essential for mouse thymocyte differentiation and TCR signaling. *Nat Immunol* **1**: 317–321
- Priatel JJ, Teh SJ, Dower NA, Stone JC, Teh HS (2002) RasGRP1 transduces low-grade TCR signals which are critical for T cell development, homeostasis, and differentiation. *Immunity* **17**: 617–627
- Priatel JJ et al (2010) RasGRP1 regulates antigen-induced developmental programming by naive CD8 T cells. *J Immunol* **184**: 666–676
- Roose JP, Mollenauer M, Gupta VA, Stone J, Weiss A (2005) A diacylglycerol-protein kinase C-RasGRP1 pathway directs Ras activation upon antigen receptor stimulation of T cells. *Mol Cell Biol* **25**: 4426–4441
- Ebisuya M, Kondoh K, Nishida E (2005) The duration, magnitude and compartmentalization of ERK MAP kinase activity: mechanisms for providing signaling specificity. *J Cell Sci* **118**: 2997–3002
- Prasad A, Zikherman J, Das J, Roose JP, Weiss A, Chakraborty AK (2009) Origin of the sharp boundary that discriminates positive and negative selection of thymocytes. *Proc Natl Acad Sci USA* **106**: 528–533
- Kortum RL, Sommers CL, Alexander CP, Pinski JM, Li W, Grinberg A, Lee J, Love PE, Samelson LE (2011) Targeted Sos1 deletion reveals its critical role in early T-cell development. *Proc Natl Acad Sci USA* **108**: 12407–12412
- Ruiz S, Santos E, Bustelo XR (2007) RasGRF2, a guanosine nucleotide exchange factor for Ras GTPases, participates in T-cell signaling responses. *Mol Cell Biol* **27**: 8127–8142
- Acuto O, Di Bartolo V, Micheli F (2008) Tailoring T-cell receptor signals by proximal negative feedback mechanisms. *Nat Rev Immunol* **8**: 699–712
- Ravichandran KS, Igras V, Shoelson SE, Fesik SW, Burakoff SJ (1996) Evidence for a role for the phosphotyrosine-binding domain of Shc in interleukin 2 signaling. *Proc Natl Acad Sci USA* **93**: 5275–5280
- Beyer T et al (2011) Integrating signals from the T-cell receptor and the interleukin-2 receptor. *PLoS Comput Biol* **7**: e1002121
- Arndt B, Krieger T, Kalinski T, Thielitz A, Reinhold D, Roessner A, Schraven B, Simeoni L (2011) The transmembrane adaptor protein SIT inhibits TCR-mediated signaling. *PLoS ONE* **6**: e23761
- Ebinu JO et al (2000) RasGRP links T-cell receptor signaling to Ras. *Blood* **95**: 3199–3203