Title

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Journal

The Journal of Immunology, 190(9)

ISSN

0022-1767

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Publication Date

2013-05-01

DOI

10.4049/jimmunol.1203561

Peer reviewed

Cutting Edge

THE JOURNAL OF IMMUNOLOGY

Cutting Edge: A Novel, Human-Specific Interacting Protein Couples FOXP3 to a Chromatin-Remodeling Complex That Contains KAP1/TRIM28

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Regulatory T cells (Tregs) play a pivotal role in the maintenance of immunological self-tolerance. Deficiency or dysfunction of Tregs leads to severe autoimmune diseases. Although the *forkhead/winged-helix* family member FOXP3 is critical for Treg differentiation and function, the molecular basis for FOXP3 function remains unclear. In this study, we identified and characterized a human-specific FOXP3-interacting protein, referred to as FIK (FOXP3-interacting KRAB domain–containing protein). FIK is highly expressed in Tregs and acts as a bridging molecule to link FOXP3 with the chromatin-remodeling scaffold protein KAP1 (TIF-1β/TRIM28). Disruption of the FOXP3–FIK–KAP1 complex in Tregs restored expression of FOXP3-target genes and abrogated the suppressor activity of the Tregs. These data demonstrate a critical role for FIK in regulating FOXP3 activity and Treg function. ***The Journal of Immunology*****, 2013, 190: 4470–4473.**

An important aspect of immune regulation is the establishment and maintenance of tolerance to self-Ags. In the periphery, this is largely accomplished by a population of CD4 T cells, referred to as regulatory T cells (Tregs), which express the transcription factor Foxp3 (1). These cells are critical for the suppression of pathological immune responses to both self- and foreign Ag, and deficiency or dysfunction of Tregs leads to severe autoimmune disease (2, 3). The development and function of Tregs are dependent on the *forkhead*/winged-helix transcription factor FOXP3 (4–6); ablation of Foxp3 in mice, as well as FOXP3 mutations in humans with immune dysfunction, polyendocrinopathy, enteropathy, X-linked syndrome, leads to the rapid onset of fatal autoimmune lymphoproliferative disease (7). In addition, ectopic expression of FOXP3 or Foxp3 in naive CD4 T cells from humans and mice, respectively, leads to the generation of T cells with suppressor activity (4, 8). Although the importance of FOXP3 in Tregs is clear, the molecular mechanism by which it controls gene expression in Tregs is largely unknown. In this article we show that, in human Tregs, a novel interacting protein couples FOXP3 to a repressive chromatin-remodeling complex that includes the adaptor KAP1/TRIM28. The interacting protein is found only in human Tregs and demonstrates a fundamental difference in the mechanism by which FOXP3 regulates transcription in human and mouse Tregs.

Materials and Methods

Yeast 2-hybrid screen

The yeast 2-hybrid screen was performed as described previously (9).

Abs

Abs were purchased from eBioscience (San Diego, CA; FOXP3, CD152, IL-2, and IFN-γ), BioLegend (San Diego, CA; FOXP3), Abcam (Cambridge, U.K.; FOXP3 and KAP1), and BD Pharmingen (San Jose, CA; CD4 and CD25). Anti-human FOXP3-interacting KRAB domain–containing protein (FIK) Ab was raised by immunizing rabbit with the peptide SKGERRQKLPRKNP (corresponding to aa 99–112 of human FIK) conjugated to FKL.

Cell culture and transfection

HEK 293T cells and Jurkat T cells were maintained as previously described (9). HEK 293T cells were transfected using *Trans*IT-LT1 Transfection Reagent (Mirus, Madison, WI). Jurkat T cells were transfected using Amaxa Cell Line Nucleofector Kit V (Lonza). CD4 T cells were isolated from PBMCs using a human Naive CD4 T Cell Isolation Kit II (Miltenyi Biotec, Auburn CA). $CD4^+CD25^-$ naive T cells and $CD4^+CD25^+$ Tregs were isolated by cell sorting.

Reverse transcription and quantitative PCR

Total RNA was isolated using TRIzol reagent and GenElute Mammalian Total RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO). Quantitative real-time PCR (qPCR) was performed, as described (9), using the following primers: human *FOXP3*, forward: 5′-TGACCAAGGCTTCATCTGTG-3′ and reverse: 5′-GAGGAACTCTGGGAATGTGC-3′; human *FIK*, forward: 5′-GTTTCCAAGCCAGAGGTGA-3′ and reverse: 5′-CCCAGATGGTAG-CGACAGC-3′; human IL-2, forward: 5′-ATTACAAGAATCCCAAACTC-3′ and reverse: 5′-ATTGCTGATTAAGTCCCT-3′; human IFN-γ, forward: 5′-ACTTCTTTGGCTTAATTCTC-3′ and reverse: 5′-TCCATTATCCGC-TACATC-3′; and human GAPDH, forward: 5′-GGATTTGGTCGTAT-TGGG-3′ and reverse: 5′-GGAAGATGGTGATGGGATT-3′.

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Received for publication January 7, 2013. Accepted for publication March 6, 2013.

This work was supported by a grant from the Juvenile Diabetes Research Foundation Cooperative Center for Cell Therapy (to S.F.Z., J.H.B., J.A.B., and J.L.R.).

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The online version of this article contains supplemental material.

Abbreviations used in this article: ChIP, chromatin immunoprecipitation; Co-IP, coimmunoprecipitation; dnFIK, dominant-negative FOXP3-interacting KRAB domain–containing protein; FIK, FOXP3-interacting KRAB domain–containing protein; qPCR, quantitative PCR; siRNA, small interfering RNA; Treg, regulatory T cell.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1203561

Coimmunoprecipitation and Western blot

Coimmunoprecipitation (Co-IP) and Western blot were performed as described previously (9).

Chromatin immunoprecipitation assays

Chromatin immunoprecipitation (ChIP) assays were performed according to the manufacturer's instructions (Upstate Biotechnology, Lake Placid, NY). The following primers were used for ChIP: human *IL-2* promoter, forward: 5′-CTCTTGCTCTTGTCCACCAC-3′ and reverse: 5′-ATCCCTCTTTGTTACATTAGCC-3′; and human IFN-γ promoter, forward: 5′-CCAGTCCTTGAATGGTGTGAAGT-3′ and reverse: 5′-TAACTAAGGTTTTGTGGCATTTGGG-3′.

Retroviral and lentiviral infection

Full-length human *FOXP3* and *FIK* were cloned into retroviral vectors pMIGR1 (*FOXP3*) or pLXSN-NGFR (*FIK*) and packaged as previously described (10). Jurkat T cells were infected by retrovirus encoding FOXP3 and/or FIK by spin infection. Full-length human FIK or dominant-negative FIK (dnFIK) was cloned into lentivirus vector pCVL.MND.SceOPT.2A.GFP (11).

RNA interference

Small interfering RNA (siRNA) oligonucleotides specific to human *FOXP3* (siRNA sequence: CACUCAAUGAGAUCUACCA[dT][dT]) and *KAP1* (siRNA sequence: GCUCUACUGGGCCAGCCA A[dT][dT]) (Sigma-Aldrich) were introduced into primary human Tregs by nucleofection using an Amaxa instrument (Lonza).

Treg-suppressive assay

$CD4^+CD25^+CD45RA^+$ primary human T cells were sort purified and transduced with lentiviral vectors, as described previously (11), or transfected with siRNA, as described above. Suppression activity was measured using CFSE-dilution assays, as described previously (12), or a BD FastImmune Regulatory T-Cell Function Kit, according to the manufacturer's instructions with minor modifications. The percentage suppression was calculated using the formula: (1 − number of effector T cell divisions in suppressed condition/number of effector T cell divisions in unsuppressed condition) × 100.

Results and Discussion

To gain an understanding of the molecular basis of FOXP3-mediated transcriptional regulation, we performed a yeast 2-hybrid screen using the amino terminal 200 aa of FOXP3 as bait and human Treg cDNA as prey. This region was chosen because we showed previously that it contained sequences required for FOXP3 to act as a transcriptional repressor (13). Among the sequences that bound to this region of FOXP3 were RORα and RORγt, which provided important insights into the role of FOXP3 in the regulation of Treg/Th17 differentiation (9, 10). In addition, a clone that encoded a protein of 113 aa was isolated, with the first 85 aa identical to those in the Kruppel family gene *ZFP90*, whereas the C-terminal 26 aa are unique (Supplemental Fig. 1A). An examination of the gene structure showed that the mRNA encoding this protein contains the first three exons of *ZFP90*, in addition to a unique sequence that is 3′ of the *ZFP90* gene, suggesting that this mRNA was generated through alternative splicing of the *ZFP90* mRNA (data not shown). The *ZNP90* sequences retained in the mRNA encode a KRAB domain (Supplemental Fig. 1A). This domain, which is present in about one third of the 300–700 human ZFPs, is one of the most widely distributed transcriptional-repression domains identified in mammals (14, 15). KRAB domains are 50–75-aa sequences consisting of two motifs, referred to as the A and B boxes. The A box recruits a corepressor complex through association with KAP1/TIF1β/TRIM28 (referred to in this article as KAP1), whereas the function of the B box remains unknown (16, 17). Based on the presence of a KRAB domain, we refer to this protein as FIK (FOXP3-interacting KRAB domain–containing protein), which becomes the third example of a novel protein generated from differential splicing of a Kruppel-family ZnF gene (18, 19). Interestingly, we were unable to find a sequence homologous with the unique C-terminal sequence of *FIK* in the mouse genome, although an ortholog of *ZFP90* was found in mouse chromosome 8 (data not shown). Thus, FIK is a human-specific FOXP3-interacting protein. Consistent with its role in Tregs, $CD4^+CD25^+$ human T cells expressed the spliced mRNA encoding *FIK* (Fig. 1A), and the ability of FIK to interact with FOXP3 was shown by Co-IP from $CD4^+CD25^+$ human T cells (Fig. 1B).

To map the interacting domains within FOXP3 and FIK, we constructed a truncation series for each and used Co-IP following transient transfection. As shown in Supplemental Fig. 1B, the proline rich N-terminal of FOXP3, especially aa 106–198, is required to interact with FIK. Interestingly, this region of FOXP3 can also interact with Eos to mediate gene silencing in Tregs (20). In addition, Li et al. (21) showed that this region of FOXP3 was important for its ability to interact with Tip60 and histone deacetylases. Taken together, these data suggest that this region of FOXP3 is critical in regulating its association with repressive chromatin-remodeling complexes, possibly through interactions involving FIK. The unique C-terminal region of FIK is necessary and sufficient for interacting with FOXP3 (Supplemental Fig. 1C).

As mentioned previously, a major feature of FIK is the presence of a KRAB domain (Supplemental Fig. 2A). As described above, this domain, found primarily in about one third of eukaryotic Kruppel-type C2H2 ZFPs, serves as a binding site for the scaffolding protein KAP1 (16, 17). Gene silencing requires the binding of the KRAB domain to the RING-B box-coiled coil domain of the corepressor KAP1. KAP1 then represses gene transcription by recruiting the histone methyltransferase SETDB1, HP1, and the NuRD histone deacetylase complex (17). The KRAB domain in FIK contains both A box and B box; both are required for it to bind to KAP1 (Supplemental Fig. 2B). These data suggest a model whereby FIK acts as a bridge between FOXP3 and a gene-silencing chromatin-remodeling complex that contains KAP1. To test this hypothesis, we first asked whether FOXP3, FIK, and KAP1 formed a complex in cells. As shown in Fig. 2A, following cotransfection of expression plasmid encoding each protein,

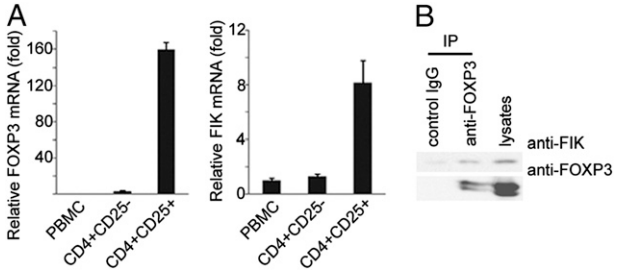

FIGURE 1. FIK is highly expressed in Tregs and physically interacts with FOXP3. (**A**) PBMCs were isolated and sorted into $CD4^+CD25^-$ naive T cells and $CD4^+CD25^+$ Tregs. Reverse transcription and qPCR were performed as described. Expression of *FOXP3* and *FIK* mRNA relative to control *GAPDH* (in triplicate wells ± SD) was determined. Transcript level in the PBMC group was considered as 1. (**B**) Cell lysates from expanded $CD4^+CD25^+$ Tregs were subject to a Co-IP experiment. Anti-human FOXP3 Ab was used as immunoprecipitation Ab. Western blots were performed to detect both FOXP3 and FIK. Data represent three or more independent experiments. Error bars indicate SD.

immunoprecipitating KAP1 (anti-FLAG), FOXP3 could be coimmunoprecipitated. We also transduced the human T cell line Jurkat, which does not express FOXP3 or FIK but does express KAP1, with retroviruses encoding these genes to determine their role in regulating IL-2 and IFN-γ expression. After 3 d, cells transduced with both retroviruses were sorted and stimulated with PMA plus ionomycin for 4 h, and IL-2 and IFN-γ expression was analyzed. As shown in Fig. 2B, FOXP3 alone can suppress IL-2 and IFN-γ expression, whereas FIK alone cannot. However, cells transduced with both genes showed a significantly decreased level of IL-2 and IFN-γ expression, consistent with a role for FIK in FOXP3 function.

We next performed a ChIP assay to determine whether the FOXP3–FIK–KAP1 complex physically binds to a target gene promoter. Human $CD4^+CD25^+$ cells were isolated and expanded in vitro. A ChIP assay was performed using Abs against FOXP3, FIK, and KAP1. As shown in Fig. 2C, we were able to immunoprecipitate all three proteins from the same site on the IL-2 promoter. Similar results were obtained using a site from the IFN-γ promoter (Fig. 2D). Taken together, these data demonstrate that FOXP3, FIK, and KAP1 can form a complex that is present on the promoters of FOXP3-repressed genes.

Next, we investigated whether blockade of FOXP3–FIK–KAP1 interaction in Tregs would affect gene expression and suppressor function in Tregs. We initially attempted to generate siRNAs to target *FIK* but were unsuccessful in finding sequences that led to demonstrable knockdown of *FIK* mRNA. We next took advantage of the fact that the C terminus of FIK was capable of binding to FOXP3 but that the entire KRAB domain was required for KAP1 interaction (Supplemental Figs. 1, 2). Using this information, we designed a form of FIK that would bind to FOXP3, but not to KAP1 (aa 75–113), and thus act as a dominant negative (dnFIK), competing with endogenous FIK for binding to FOXP3. A lentiviral vector encoding this fragment linked to GFP via a 2A sequence was used to transduce human $CD4^+CD25^+$ Tregs. Cells expressing dnFIK showed increased IL-2 and IFN-γ production following stimulation, two genes known to be repressed by FOXP3 (Fig. 3A). However, expression of genes that were shown to be positively

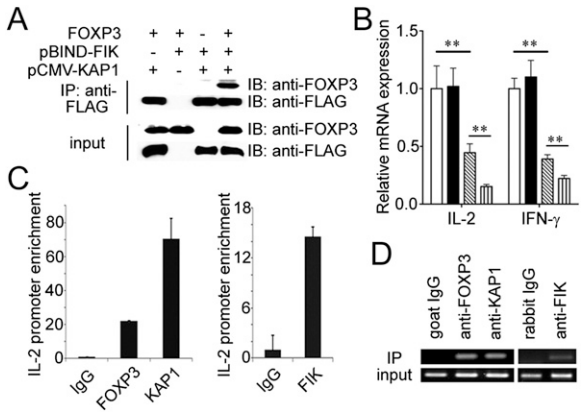

FIGURE 2. FOXP3, FIK, and KAP1 form a transcription-suppressive complex in Tregs. (**A**) HEK 293T cells were transfected with plasmids, as indicated. Co-IP experiments were performed, as described, using anti-FLAG Ab. (**B**) Jurkat T cells were infected with retrovirus encoding FOXP3 and FIK, as described. After 3 d, double-positive cells were sorted and stimulated. *IL-2* and *IFN-γ* transcription relative to *GAPDH* was quantified by qPCR analysis. Open bar: MIGR1+NGFR; filled bar: MIGR1+FIK; hatched bar: FOXP3+NGFR; bar represents FOXP3+FIK. Transcript level in the control group infected with MIGR1 plus NGFR was set as 1. (**C** and **D**) ChIP assays were performed as described in *Materials and Methods*. Goat anti-human FOXP3, goat anti-human KAP1, and rabbit anti-human FIK Abs were used to pull down the protein–DNA complexes, followed by qPCR analysis (C) or gel electrophoresis (D) using primers specific to human *IL-2* (C) and *IFN-γ* (D) promoter. Data are mean ± SD and represent three independent experiments. $^{**}p < 0.01$.

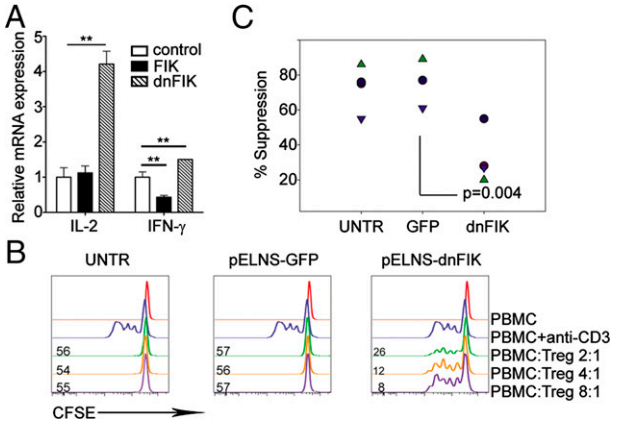

FIGURE 3. FOXP3–FIK interaction is essential for Treg function. $CD4^+CD25^+$ Tregs were maintained in X-VIVO medium with anti-CD3/CD28 beads plus 300 U/ml IL-2. These cells were infected by lentivirus encoding full-length FIK or dnFIK or corresponding control virus. GFP^+ cells were sorted after 4 d and analyzed. (**A**) Sorted GFP^+ cells were stimulated with anti-CD3/CD28 beads for 4 h. Total RNA was reverse transcribed, and qPCR was performed to determine *IL-2* and *INF-γ* expression. $^{**}p < 0.01$. (**B**) Sorted GFP^+ cells were mixed with CFSE-labeled PBMCs in the presence of anti-CD3 beads and cultured for 4 d. CFSE dilution of $CD8^+$ cells was assessed by flow cytometry. UNTR represents untreated Tregs; pELNS-GFP represents Tregs infected by control virus, and pELNS-dnFIK represents Tregs infected by virus encoding dnFIK. The number beside the peak represents the suppressive activity of each group. (**C**) Summary of four independent suppressive assays. Data are mean ± SD and represent three independent experiments.

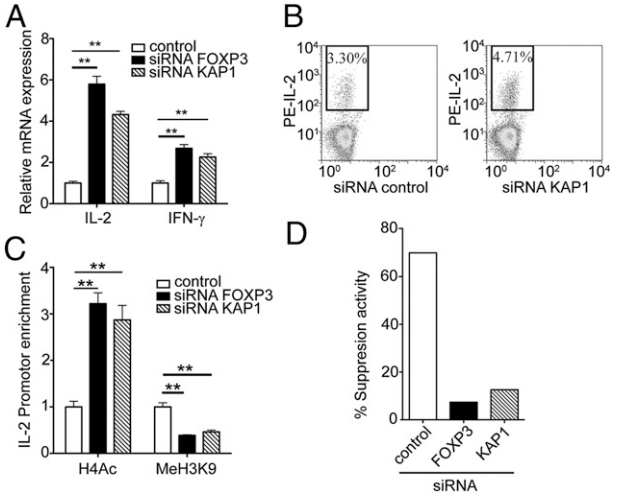

FIGURE 4. KAP1 is required for FOXP3 function. $CD4^+CD25^+$ Tregs were transfected with siRNA specific to human *FOXP3* or *KAP1* using Amaxa Human T Cells Transfection Kit. Cells were harvested after 3–4 d. (**A**) *IL-2* and *IFN-γ* expression were determined by qPCR normalized to *GAPDH* expression. (**B**) IL-2 expression was also analyzed by intracellular staining. (**C**) ChIP assays were performed, as described. Rabbit anti-histone H3K9 (ab8898; Abcam) and rabbit anti–acetyl-histone H4 (17-630; Millipore) were used to pull down the protein–DNA complexes, followed by qPCR analysis using primers specific to human *IL-2* promoter. (**D**) Suppressive assay was performed using a BD FastImmune Regulatory T-Cell Function Kit, according to the manufacturer's instructions with minor modifications. Data are mean ± SD and represent three independent experiments. $^{**}p < 0.01$.

regulated by FOXP3 (CTLA-4 and CD25) was unaffected by dnFIK (data not shown).

We next determined whether inhibiting FOXP3–KAP1 interactions affected Treg function. For these studies, we again transduced activated human $CD4^+CD25^+$ T cells with the dnFIK lentivirus and sorted GFP^+ cells. The infected Tregs were cultured at various ratios with CFSE-labeled PBMCs and stimulated with anti-CD3 beads. Proliferation was measured by CFSE dilution of $CD8^+$ T cells. We found that Tregs expressing dnFIK had a dramatically reduced ability to suppress the proliferation of conventional CD8 T cells (Fig. 3B, 3C), showing that FOXP3–FIK–KAP1 interactions play an important role in human Treg function.

These studies were extended to directly determine the role of KAP1 in human Tregs, targeting *FOXP3* and *KAP1* with siRNAs. Human $CD4^+CD25^+$ T cells were expanded in vitro with anti-CD3/CD28 beads plus 300 U/ml IL-2, as described (22). These expanded Tregs were treated with siRNA specific to human *FOXP3* and *KAP1*; after 3 d, these cells were further stimulated with PMA plus ionomycin, and cytokine expression was assessed. Knockdown of FOXP3 or KAP1 in $CD4^+CD25^+$ Tregs resulted in increased expression of IL-2 and IFN-γ (Fig. 4A, 4B). Concomitant with this increased expression, the *IL-2* promoter displayed an increase in epigenetic marks consistent with open chromatin (acetylated histone H4) and decreased trimethyl histone H3K9, indicative of closed chromatin (Fig. 4C). Finally, we determined the ability of the *FOXP3*- and *KAP1*-knockdown Tregs to suppress T cell proliferation in vitro. Both cell populations had a dramatic reduction in suppressor function in vitro (Fig. 4D). These data demonstrate that KAP1 is important for FOXP3 and Treg function.

KAP1 is a ubiquitously expressed multifunctional protein that was shown to be a critical factor for chromatin stability in both humans and mice (17). Recently, Chikuma et al. (23) reported that mouse T cells require Kap1 for proper cell-cycle progression in response to TCR stimulation. In addition, they showed that mice with T cell–specific deletion of KAP1 develop spontaneous autoimmunity (23). However, Tregs from these mice display equivalent suppressor function in vitro as do their wild-type counterparts, suggesting that the defect in these animals is in the effector T cell compartment. These data are consistent with our model, because mice lack an FIK ortholog and, thus, cannot couple Kap1 to Foxp3. Further support comes from an exhaustive analysis of Foxp3-interacting proteins that failed to identify Kap1 (24). Taken together, these data suggest that the mechanism of transcriptional repression regulated by FOXP3/Foxp3 in human and mouse Tregs differs.

Acknowledgments

We thank Drs. J. Hamerman and D. Campbell (Benaroya Research Institute) for comments on the manuscript and Sylvia McCarty for help preparing the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sakaguchi, S. 2000. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* 101: 455–458.
2. Bluestone, J. A., and A. K. Abbas. 2003. Natural versus adaptive regulatory T cells. *Nat. Rev. Immunol.* 3: 253–257.
3. Shevach, E. M. 2000. Regulatory T cells in autoimmmunity. *Annu. Rev. Immunol.* 18: 423–449.
4. Hori, S., T. Nomura, and S. Sakaguchi. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299: 1057–1061.
5. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4: 330–336.
6. Khattri, R., T. Cox, S. A. Yasayko, and F. Ramsdell. 2003. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* 4: 337–342.
7. Ziegler, S. F. 2006. FOXP3: of mice and men. *Annu. Rev. Immunol.* 24: 209–226.
8. Allan, S. E., L. Passerini, R. Bacchetta, N. Crellin, M. Dai, P. C. Orban, S. F. Ziegler, M. G. Roncarolo, and M. K. Levings. 2005. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. *J. Clin. Invest.* 115: 3276–3284.
9. Du, J., C. Huang, B. Zhou, and S. F. Ziegler. 2008. Isoform-specific inhibition of ROR alpha-mediated transcriptional activation by human FOXP3. *J. Immunol.* 180: 4785–4792.
10. Zhou, L., J. E. Lopes, M. M. Chong, I. I. Ivanov, R. Min, G. D. Victora, Y. Shen, J. Du, Y. P. Rubtsov, A. Y. Rudensky, et al. 2008. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. *Nature* 453: 236–240.
11. Plesa, G., L. Zheng, A. Medvec, C. B. Wilson, C. Robles-Oteiza, N. Liddy, A. D. Bennett, J. Gavarret, A. Vuidepot, Y. Zhao, et al. 2012. TCR affinity and specificity requirements for human regulatory T-cell function. *Blood* 119: 3420–3430.
12. Golovina, T. N., T. Mikheeva, M. M. Suhoski, N. A. Aqui, V. C. Tai, X. Shan, R. Liu, R. R. Balcarcel, N. Fisher, B. L. Levine, et al. 2008. CD28 costimulation is essential for human T regulatory expansion and function. *J. Immunol.* 181: 2855–2868.
13. Lopes, J. E., T. R. Torgerson, L. A. Schubert, S. D. Anover, E. L. Ocheltree, H. D. Ochs, and S. F. Ziegler. 2006. Analysis of FOXP3 reveals multiple domains required for its function as a transcriptional repressor. *J. Immunol.* 177: 3133–3142.
14. Collins, T., J. R. Stone, and A. J. Williams. 2001. All in the family: the BTB/POZ, KRAB, and SCAN domains. *Mol. Cell. Biol.* 21: 3609–3615.
15. Urrutia, R. 2003. KRAB-containing zinc-finger repressor proteins. *Genome Biol.* 4: 231.
16. Peng, H., G. E. Begg, D. C. Schultz, J. R. Friedman, D. E. Jensen, D. W. Speicher, and F. J. Rauscher, III. 2000. Reconstitution of the KRAB-KAP1 repressor complex: a model system for defining the molecular anatomy of RING-B box-coiled-coil domain-mediated protein-protein interactions. *J. Mol. Biol.* 295: 1139–1162.
17. Iyengar, S., and P. J. Farnham. 2011. KAP1 protein: an enigmatic master regulator of the genome. *J. Biol. Chem.* 286: 26267–26276.
18. Li, Z., D. Wang, X. Na, S. R. Schoen, E. M. Messing, and G. Wu. 2003. The VHL protein recruits a novel KRAB-A domain protein to repress HIF-1alpha transcriptional activity. *EMBO J.* 22: 1857–1867.
19. Oh, H. J., Y. Li, and Y. F. C. Lau. 2005. Sry associates with the heterochromatin protein 1 complex by interacting with a KRAB domain protein. *Biol. Reprod.* 72: 407–415.
20. Pan, F., H. Yu, E. V. Dang, J. Barbi, X. Pan, J. F. Grosso, D. Jinasena, S. M. Sharma, E. M. McCadden, D. Getnet, et al. 2009. Eos mediates Foxp3-dependent gene silencing in CD4+ regulatory T cells. *Science* 325: 1142–1146.
21. Li, B., A. Samanta, X. Song, K. T. Iacono, K. Bembas, R. Tao, S. Basu, J. L. Riley, W. W. Hancock, Y. Shen, et al. 2007. FOXP3 interactions with histone acetyltransferase and class II histone deacetylases are required for repression. *Proc. Natl. Acad. Sci. USA* 104: 4571–4576.
22. Putnam, A. L., T. M. Brusko, M. R. Lee, W. Liu, G. L. Szot, T. Ghosh, M. A. Atkinson, and J. A. Bluestone. 2009. Expansion of human regulatory T-cells from patients with type 1 diabetes. *Diabetes* 58: 652–662.
23. Chikuma, S., N. Suita, I. M. Okazaki, S. Shibayama, and T. Honjo. 2012. TRIM28 prevents autoinflammatory T cell development in vivo. *Nat. Immunol.* 13: 596–603.
24. Rudra, D., P. deRoos, A. Chaudhry, R. E. Niec, A. Arvey, R. M. Samstein, C. Leslie, S. A. Shaffer, D. R. Goodlett, and A. Y. Rudensky. 2012. Transcription factor Foxp3 and its protein partners form a complex regulatory network. *Nat. Immunol.* 13: 1010–1019.