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Bacterial Toxins Induce Sustained mRNA Expression of the Silencing Transcription Factor *klf2* via Inactivation of RhoA and Rhophilin 1[▽]

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Yersiniae bearing the *Yersinia* virulence plasmid pYV impact the transcriptome of J774A.1 macrophage-like cells in two distinct ways: (i) by suppressing, in a *Yersinia* outer protein P (YopP)-dependent manner, the induction of inflammatory response genes and (ii) by mRNA induction of the silencing transcription factor *klf2*. Here we show that *klf2* induction by *Yersinia enterocolitica* occurs in several cell lines of macrophage and squamous and upper gastrointestinal epithelial origin as well as in bone marrow-derived dendritic cells. Several strains of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are equally effective as *Y. enterocolitica* in inducing *klf2* expression. Screening of mutant strains or incubation with recombinant toxins identified the rho-inactivating toxins YopT from *Yersinia* spp., ExoS from *Pseudomonas aeruginosa*, EDIN-B from *Staphylococcus aureus*, and C3bot from *Clostridium botulinum* as bacterial inducers of *klf2* mRNA. *klf2* mRNA induction by these toxins does not require de novo protein synthesis. Serum response factor or actin depolymerization does not seem to be involved in regulating *klf2* expression in response to bacterial infection. Instead, short hairpin RNA-mediated inactivation of RhoA and its effector rhophilin 1 is sufficient to induce long-term *klf2* expression. Thus, bacteria exploit the RhoA-rhophilin signaling cascade to mediate sustained expression of the immunosuppressive transcription factor *klf2*.

The genus *Yersinia* comprises three species that are pathogenic to humans and rodents: *Yersinia pestis*, the etiologic agent of plague, causes systemic and life-threatening disease; and *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* are enteropathogens which cause gastrointestinal diseases, including mesenterial lymphadenitis, and, rarely, systemic infections. *Yersinia* is endowed with a unique capacity to withstand the host attack by injecting antihost effector proteins (*Yersinia* outer proteins [Yops]) into professional phagocytes via a type III protein secretion system (TTSS) (11) Both the TTSS and Yops are encoded by a 70-kb virulence plasmid (pYV) that is common to all pathogenic *Yersinia* spp. The six established effector proteins interfere with distinct signaling pathways, resulting in paralysis of phagocyte function. In particular, there are three different effector proteins that interfere with signaling from small GTPases. The protein kinase YopO (YpkA in *Y. pseudotuberculosis*) physically interacts with RhoA and Rac-1 (7) (although the functional relevance of this interaction has not been shown to date) and inhibits Gαq signaling (42). YopE is a GTPase activating protein (GAP) for Rho, Rac, and Cdc42, accelerating GTP hydrolysis and converting these proteins into the inactive, GDP-bound form (68). YopT, a cysteine protease, inactivates RhoA by cleavage adjacent to a prenylated cysteine located near the carboxy terminus, resulting in membrane release and cytoplasmic redistribution of RhoA (60). However, some studies also showed some effect of YopT on Rac and Cdc42 in biochemical assays performed in vitro (61), but after infection of living cells, YopT seems to act mainly on RhoA (2).

Recent large-scale gene expression studies identified a number of genes induced by the action of *Yersinia* Yops in macrophages (24, 54) and epithelial cells (8). We and others (43) have identified the transcription factor *klf2* as one gene with particularly pronounced induction in host cells in response to, for example, infection with *Yersinia enterocolitica* (24), *Pseudomonas aeruginosa* (44), or *Staphylococcus aureus* (40). However, in most of these cases, the causative bacterial toxins have not been identified, with the exception of *Clostridium botulinum* C3 toxin (58).

KLF2 (formerly termed LKLF, for lung Kruppel-like factor) belongs to the KLF zinc finger family of transcription factors. KLF family members, such as KLF6, play a role in many cellular processes, including apoptosis, proliferation, differentiation, and development. KLF6, a tumor suppressor, has been shown to be induced by bacterial toxins (43). KLF2 has been identified by virtue of its homology with Eklf and is expressed primarily in the adult lung and, to a much lower extent, in the spleen (5). $klf2^{-/-}$ mice die between 11.5 and 13.5 days postconception, from severe hemorrhage (69) due to abnormal tunica media formation (32). However, experiments employing chimeric mice provided evidence that Klf2 is essential for normal lung development (70). It was shown recently that Klf2 is upregulated upon maturation of single positive T cells and that expression of *klf2* is required to program the quiescent state of single positive T cells. $klf2^{-/-}$ T cells have a spontaneously activated phenotype and are rapidly eliminated by Fas ligand-induced apoptosis (32). Consistently, inducible expression of *klf2* in Jurkat T cells is sufficient to induce a quiescent phenotype characterized by reduced proliferation, reduced protein

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TABLE 1. Cell lines used in this study

Cell line	Tissue of origin	Source (strain) or reference
J774A.1	Mouse macrophages	ATCC TIB-67
P388D1	Mouse macrophages	ATCC CCL-46
RAW264.7	Mouse macrophages	ATCC TIB-71
ANA-1	Mouse macrophages	12
U-937	Human histiocytic lymphoma	ATCC CRL-1593.2
Jurkat	Human T-cell acute lymphoblastic leukemia	56
Daudi	Human B-cell lymphoma	ATCC CCL-213
HeLa	Human cervix adenocarcinoma	ATCC CCL-2
AGS	Human gastric adenocarcinoma	ATCC CRL-1739
MKN-28	Human gastric adenocarcinoma	28

synthesis, and decreased surface expression of activation markers (10). Moreover, in endothelial cells, *klf2* is induced in response to shear stress (14) and inhibits expression of cell adhesion molecules in response to proinflammatory cytokines (59). Together, these data show that KLF2 has broadly inactivating functions in an ontogenetically diverse collection of cell types.

Here we further investigate bacterial mechanisms to induce mRNA expression of *klf2* in host cells. Specifically, we asked if the ability to induce *klf2* mRNA is specific for macrophage-like cells, if it is specific to *Yersinia*, which bacterial proteins are capable of inducing *klf2* mRNA, and how exactly bacterial toxins mediate sustained *klf2* expression.

MATERIALS AND METHODS

Bacteria and mutants. Cell lines and bacteria employed are listed in Tables 1 and 2. For generation of *P. aeruginosa exoS* mutants, the *exoS* gene of strain PAO1 was subcloned into pCR2.1-Topo (Invitrogen), leading to the plasmid pTexoS (30). The Ω fragment from plasmid pHP45-Ω, conferring resistance to streptomycin and spectinomycin (48), was ligated into the unique HincII restriction site of *exoS*. The disrupted *exoS* gene was cloned into the mobilizable suicide vector pEX18Ap, carrying the counterselectable *sacB* marker (23). The resulting plasmid, pEXexoS::Ω, was conjugated into strains *P. aeruginosa* PAO1 and PAK by triparental mating with *Escherichia coli*(pRK2013). Transconjugants were selected on Luria-Bertani (LB) agar plates containing 500 μg/ml carbenicillin. Sucrose-resistant *exoS* mutant strains with a carbenicillin-sensitive and streptomycin-resistant phenotype were selected on *Pseudomonas* isolation agar containing 5% sucrose. PAO1 and PAK *exoS* mutants were confirmed by Southern hybridization (data not shown) and Western blotting using polyclonal ExoS antibody. Recombinant six-His-tagged EDIN-B toxin was a gift from M. Aepfelbacher (University of

TABLE 2. Bacterial strains used in this study

Strain	Description	Source or reference
Yersinia strains		
WA-C	Plasmidless derivative of WA(pYV)	21
WA(pYV)	*Y. enterocolitica* O:8	21
8081	*Y. enterocolitica* O:8	63
WA(pTTS, pYopT)	WA-C derivative translocating YopT only	66
WA(pTTS, pYopT C139S)	WA-C derivative translocating a catalytically inactive YopT	35, 60
WA(pTTS, pYopE)	WA-C derivative translocating YopE only	66
WA(pTTS, pYopO)	WA-C derivative translocating YopO only	66
WA(pYVΔYopT)	YopT-deficient WA(pYV) derivative	67
534	*Y. pseudotuberculosis* clinical isolate	Max von Pettenkofer Institute clinical strain collection
591	*Y. pseudotuberculosis* clinical isolate	Max von Pettenkofer Institute clinical strain collection
601	*Y. pseudotuberculosis* clinical isolate	Max von Pettenkofer Institute clinical strain collection
686	*Y. pseudotuberculosis* clinical isolate	Max von Pettenkofer Institute clinical strain collection
Other strains		
Enteropathogenic *E. coli* E2348/96		19
Enterohemorrhagic *E. coli* O157:EDL933		19
Enterohemorrhagic *E. coli* 413/89-1		74
Citrobacter rodentium		17
Citrobacter freundii		ATCC 29219
Listeria monocytogenes EGD		ATCC BAA-697
Helicobacter pylori P12		55
Helicobacter pylori TIGR		65
Campylobacter jejuni C31	Clinical isolate	R. Haas, Munich, Germany
Campylobacter jejuni C63		20
Campylobacter jejuni C64		20
Hafnia alvei 10790		29
Shigella flexneri M90T		53
Pseudomonas aeruginosa PAO1		64
Pseudomonas aeruginosa PAK		16
Pseudomonas aeruginosa PA103		16
PAO1ΔpcrD	TTSS-deficient derivative of PAO1	26
PAO1ΔexoS	*exoS*-deficient derivative of PAO1	This study
PAKΔpcrD	TTSS-deficient derivative of PAK	26
PAKΔexoS	*exoS*-deficient derivative of PAK	This study
Staphylococcus aureus 57	Clinical isolate	M. Aepfelbacher, Hamburg, Germany
Staphylococcus aureus Newman		ATCC 25904
Salmonella enterica serovar Typhimurium SL1344		25

Hamburg, Germany), and recombinant *C. botulinum* C3 toxin was a gift from Stefan Linder (Ludwig Maximilians University, Munich, Germany). Recombinant six-His-tagged SycE protein was a gift from G. Wilharm (University of Munich, Germany).

Cell culture and bacterial infection. Dendritic cells (DCs) were generated from bone marrows of C57BL/6 mice as described previously (37). Cell lines were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum. Unless noted otherwise, stationary-phase overnight bacterial cultures were diluted 1:10 in fresh LB medium and grown to an optical density at 600 nm of 0.5 to 0.6 (about 6×10^8 CFU/ml). Cells were infected at multiplicities of infection of 100:1 and 10:1. *Campylobacter jejuni* strains were grown on Columbia blood agar plates (Becton Dickinson) in 5% CO_2 and restreaked every 48 h. For infections, bacteria were harvested from agar plates and diluted in Dulbecco's modified Eagle's medium. *Helicobacter pylori* strains were grown on GC agar plates (Doenitz Prolab, Augsburg, Germany) supplemented with 8% horse serum, a complex vitamin mixture, vancomycin (10 mg/liter), trimethoprim (5 mg/liter), and nystatin (1 mg/liter) and were incubated for 1 to 2 days in a microaerobic atmosphere (85% N_2, 10% CO_2, 5% O_2) at 37°C. Stationary-phase *Yersinia* cultures grown overnight at 27°C were diluted 1:10 in fresh LB medium and shifted to 37°C for 2 h, and infections were performed as described above. Recombinant EDIN-B and SycE were applied at 60 μg/ml overnight, and recombinant *C. botulinum* C3 toxin was applied at 25 μg/ml overnight. In some experiments, cells were incubated for 2 hours to overnight with 1 to 10 μM Rho kinase inhibitor H1152 (Calbiochem).

Generation of lentivirus and transduction of AGS cells. The BLOCK-iT lentiviral RNA interference (RNAi) expression system (Invitrogen) was used according to the manufacturer's specifications. Briefly, short hairpin RNA (shRNA) oligonucleotides were selected with BLOCK-iT RNAi Designer software from Invitrogen. To avoid sequence homology with other genes, BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/) was performed. The following target mRNA sequences were used to design shRNAs (starting positions are noted in parentheses): *rhoA* (687), GCCGGTGAAACCTGAAGAAGG; *rhoB* (841), GCATCCAAGCCTACGACTACC; *rhoC* (630), GCCTCCAGGTCCGC AAGAACA; *rhpn1* (384), GCTGATCTCAGTGCACTTTGG; *cit* (685), GCG TTCATCTGATGGGATACG; *rok2* (2228), GCAGCTGGAATCTAACAA TAG; and *ktn1* (1390), GCAGATGAAGTTTCAGCAAGT. A random-sequence shRNA was used as a control. Oligonucleotides were annealed and then cloned into the entry vector pENTR/U6. TOP10 competent *E. coli* cells were transformed, and colonies were selected on LB plates containing 50 μg/ml kanamycin. After sequence verification, the U6 RNAi cassette was transferred by LR recombination to the pLENTI6/BLOCK-iT-DEST plasmid. A lentiviral stock was produced by cotransfection of 293FT cells with the resulting expression construct and ViraPower packaging mix, using the FuGene 6 transfection reagent (Roche, Mannheim, Germany). At 48 h posttransfection, viral supernatants were harvested and filtered. AGS cells were infected with lentiviruses containing the packaged shRNAs. Gene transfer was detected using a lentiviral vector containing the *gfp* marker gene. Stable cells were generated by selection with 10 μg/ml blasticidin for 10 days.

Real-time reverse transcription-PCR. Total cellular RNA was isolated with Trizol RNA isolation reagent (Invitrogen) according to the manufacturer's recommendations. After random-hexamer-primed first-strand cDNA synthesis (Superscript II; Invitrogen), real-time PCR was performed in an ABI Prism 7000 sequence detection system (Applied Biosystems) (with detection of *hprt*, *klf2*, *rhoA*, *rhoB*, *rhoC*, *rok2*, *rhpn1*, *cit*, and *ktn1*). Amplification was done for 40 cycles, using an initial denaturation at 95°C for 10 min followed by cycles of 95°C for 15 s and 60°C for 1 min. Primers and fluorescent probes for murine *klf2* and *hprt* were described previously (24). Human *klf2* and *hprt* PCR assay kits were purchased from Applied Biosystems (Assays-on-Demand). A Universal ProbeLibrary (Roche, Mannheim, Germany) was used in combination with the Universal ProbeLibrary assay design center to design specific primers and probes for *rhoA*, *rhoB*, *rhoC*, *rhpn1*, *rok*, *cit*, and *ktn1*. Gene expression levels were recorded relative to the *hprt* housekeeping control as follows: $E = 2^{-\Delta C}T$(E = gene expression value; ΔC_T = difference in crossing points between threshold cycles for *hprt* and the gene of interest). All PCR experiments were performed at least four times, and standard deviations were calculated and displayed as error bars. For graphical display, the maximum or *Y. enterocolitica*-elicited *klf2* mRNA expression value in every graph was given an arbitrary value of 10, and the remaining values and standard deviations were scaled accordingly, graphwise. Statistical analysis was performed with Student's *t* test, as implemented in Microsoft Excel, considering *P* values of ≤0.05 statistically significant.

RESULTS

***Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* induce *klf2* mRNA in macrophages.** To confirm and extend our previous analyses (24), we first evaluated whether bacteria other than *Yersinia enterocolitica* were able to induce *klf2* mRNA expression in J774A.1 cells (Fig. 1). We first examined a panel of clinical *Y. pseudotuberculosis* isolates, which demonstrated that these strains are also able to induce *klf2* mRNA expression in host cells (Fig. 1A). Next, we screened a panel of phylogenetically diverse bacterial strains, many of whom are known to possess systems for delivery of bacterial proteins into the cytoplasm of host cells (Fig. 1B). In addition to *Yersinia*, *Pseudomonas aeruginosa* strains PAO1 and PAK, but not strain PA103, and *Staphylococcus aureus* strain 57, but not strain Newman, induced *klf2* mRNA. *Salmonella enterica* serovar Typhimurium strain SL1344 induced only low levels of *klf2* mRNA in J774A.1 cells. No significant *klf2* induction could be demonstrated for strains of enterohemorrhagic *Escherichia coli*, enteropathogenic *Escherichia coli*, *Citrobacter* spp., *Listeria monocytogenes*, *Helicobacter pylori*, *Campylobacter jejuni*, *Hafnia alvei*, or *Shigella flexneri.* Similar results were obtained with multiplicities of infection of 10:1 and 100:1 and after 2 and 4 h of infection (not shown).

YopT from *Y. enterocolitica*, ExoS from *P. aeruginosa*, EDIN-B from *S. aureus*, and C3 toxin from *C. botulinum* induce *klf2* mRNA expression. To identify the bacterial protein responsible for *klf2* induction, we screened several mutant strains of *Y. enterocolitica* and *P. aeruginosa* for *klf2* induction in host cells. As displayed in Fig. 2A, translocation of YopT alone induced *klf2* mRNA expression, while the *yopT* deletion mutant WA(pYVΔYopT) did not induce significant *klf2* mRNA levels in J774A.1 cells. A catalytically inactive YopT C139S mutant or strains secreting only YopE or YopO proteins were not able to induce *klf2* mRNA expression.

For *P. aeruginosa*, strains PAO1 and PAK, but not strain PA103, induced *klf2* mRNA in J774A.1 cells. *klf2* induction was dependent on the TTSS, as *pcrD* mutant strains PAO1ΔpcrD and PAKΔpcrD, which harbor an impaired TTSS, were not able to induce *klf2* mRNA anymore (Fig. 2B). Together, these findings suggest ExoS, a type III secreted toxin produced by strains PAO1 and PAK, but not by strain PA103, as a likely candidate mediating *klf2* induction. Consistently, *exoS* deletion mutants PAO1ΔexoS and PAKΔexoS were no longer able to induce *klf2* mRNA (Fig. 2B).

For *S. aureus*, strain 57 (a virulent clinical isolate [13]) induced high levels of *klf2* mRNA in J774A.1 cells, while the *S. aureus* reference strain Newman did not. One important difference is that strain 57, but not Newman, produces EDIN-B, a C3-like ADP-ribosyltransferase which modifies Rho GTPases (M. Aepfelbacher, unpublished observations). We incubated J774A.1 cells overnight with purified recombinant EDIN-B, and this treatment indeed resulted in the induction of high levels of *klf2* mRNA compared to treatment with an irrelevant protein (the *Y. enterocolitica* YopE chaperone SycE) (Fig. 2C). This finding could be confirmed by using the prototypical Rho-specific ADP ribosyltransferase, C3 toxin from *C. botulinum* (4), which also induced high levels of *klf2* mRNA in host cells (Fig. 2C).

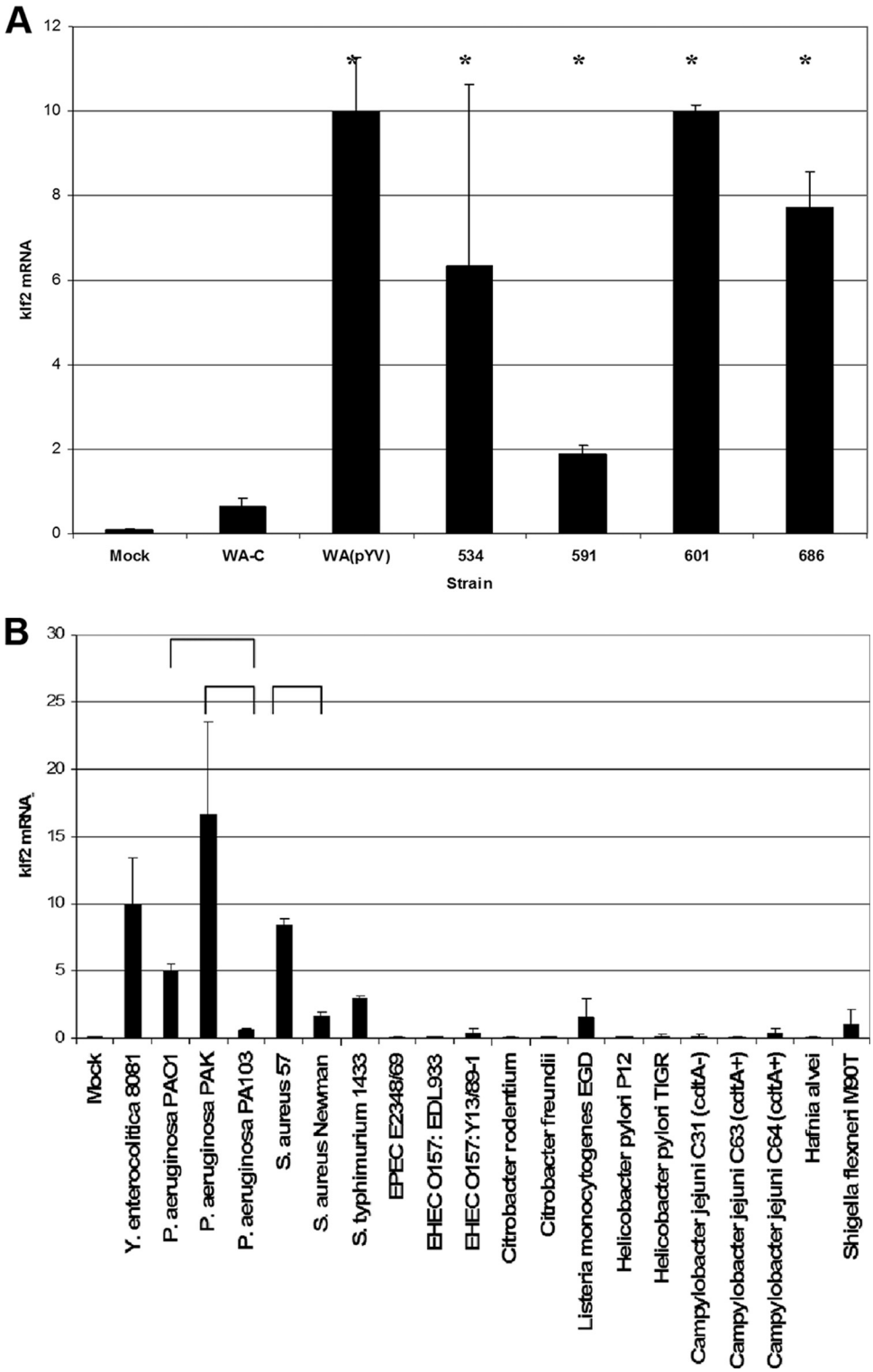

FIG. 1. *klf2* mRNA induction in J774A.1 cells by different gram-positive and gram-negative bacteria (detailed in Table 2). The *Y. enterocolitica*-elicited *klf2* level was scaled to an arbitrary value of 10. Levels of *klf2* mRNA were recorded relative to the HPRT housekeeping control. Error bars represent 1 standard deviation from the mean. Mock, uninfected cells. Horizontal brackets or asterisks indicate statistically significant (*t* test; $P \leq 0.05$) differences in *klf2* mRNA expression levels. (A) *klf2* induction by different clinical isolates of *Y. pseudotuberculosis*. (B) *klf2* induction by a collection of phylogenetically diverse bacteria.

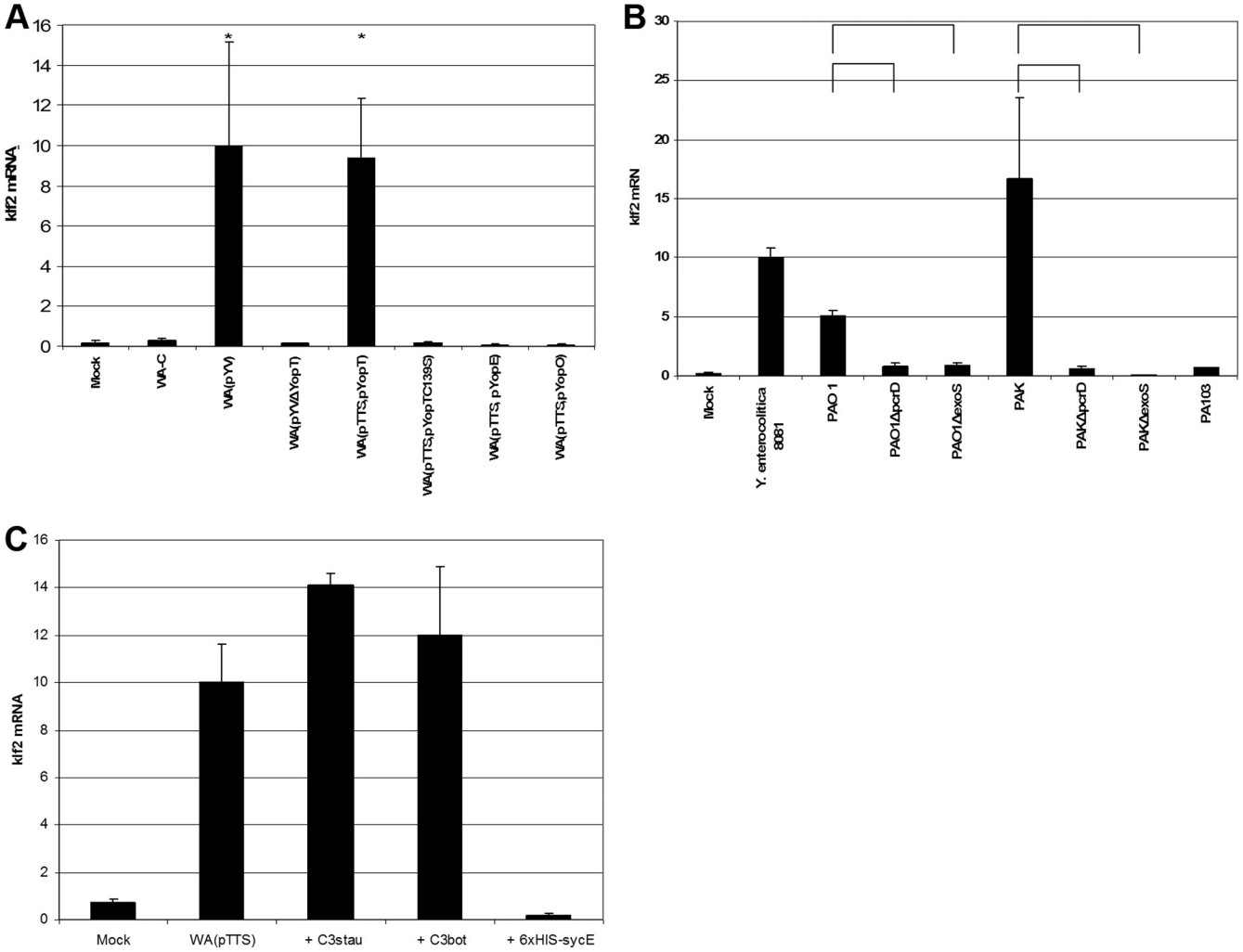

FIG. 2. Identification of bacterial *klf2*-inducing proteins. (A) *klf2* mRNA expression in J774A.1 cells after infection with different *Y. enterocolitica* mutant strains (detailed in Table 2). The graphical display is as described in the legend to Fig. 1. *, statistically significant (*t* test; $P \leq 0.05$) induction of *klf2* mRNA compared to infection with strain WA-C. (B) *klf2* mRNA levels in J774A.1 cells after infection with different *P. aeruginosa* wild-type and mutant strains (see Table 2). The graphical display is as described in the legend to Fig. 1. Horizontal brackets indicate statistically significant (*t* test; $P \leq 0.05$) differences in *klf2* mRNA expression levels. (C) *klf2* induction by recombinant EDIN-B or *C. botulinum* C3 toxin in J774A.1 cells. The graphical display is as described in the legend to Fig. 1. sycE, irrelevant protein used as negative control.

YopT induces *klf2* mRNA in cell lines of macrophage and epithelial origin. We next wondered whether induction of *klf2* mRNA upon bacterial infection could occur in cells other than J774A.1 cells. We tested a panel of mouse and human cell lines of macrophage, lymphocyte, and epithelial origin for *klf2* induction upon infection with *Y. enterocolitica* (Fig. 3). *klf2* mRNA was induced in macrophage-like (J774A.1, RAW264.7, P388D.1, and Ana-1), squamous epithelial (HeLa), and gastric epithelial (AGS and MKN-28) cells by strain WA(pTTS, pYopT). We also found *klf2* mRNA expression induced in bone marrow-derived DCs (Fig. 3). Thus, YopT is the only effector Yop required to induce *klf2* in macrophage/DC and epithelial cell lines, although other bacterial components may also contribute. In contrast, B (Daudi) or T (Jurkat) lymphocytes or histiocytes (U937) did not induce *klf2* mRNA in response to *Y. enterocolitica* (Fig. 3). It was shown earlier, however, that Yops are effectively translocated into these cells (9, 73). We concluded that YopT-mediated induction of *klf2* mRNA expression takes place mainly in macrophages, DCs, and epithelial cells.

shRNA-mediated knockdown of RhoA induces *klf2* mRNA expression. As stated above, all bacterial toxins identified to induce *klf2* mRNA expression interfere with small GTPases of the rho family, with the C3 toxins interacting specifically with RhoA, RhoB, and RhoC (4). To identify the Rho protein responsible for *klf2* induction, we generated stable shRNA-expressing cell lines for each of the Rho proteins individually. As shown in Fig. 4A to C, *rhoA*, *rhoB*, and *rhoC* shRNA-expressing cells showed substantial downregulation of the corresponding mRNA levels relative to those in control shRNA-expressing cells. However, *rhoB* and *rhoC* shRNAs also had modest effects on *rhoA* mRNA expression levels: *rhoB* shRNA resulted in a 62% reduction of the *rhoA* expression level, and *rhoC* shRNA resulted in a 68% reduction. This problem oc-

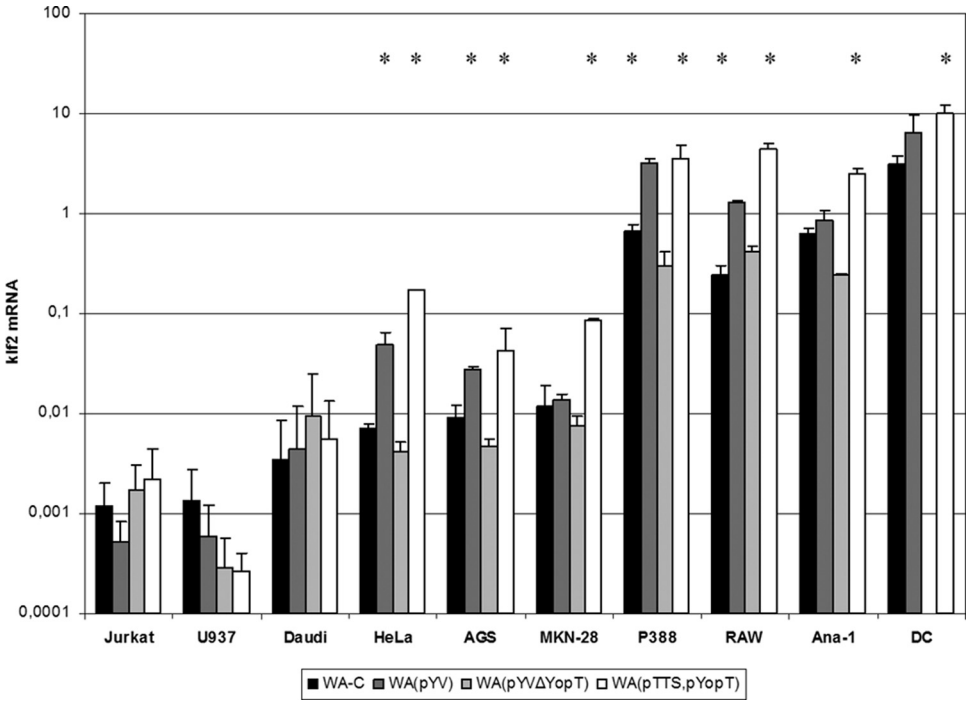

FIG. 3. *klf2* mRNA levels induced by wild-type *Y. enterocolitica* and YopT mutants in cell lines derived from different human or mouse tissues. The graphical display is as described in the legend to Fig. 1. Note the logarithmic scale. *, statistically significant (*t* test; $P \leq 0.05$) induction of *klf2* mRNA compared to infection of the respective cell line with strain WA-C. DC, mouse bone marrow-derived DCs.

curred with several different shRNA constructs. As displayed in Fig. 4D, however, *klf2* mRNA expression was highest in *rhoA* shRNA-transduced cells, while *rhoB* shRNA- and *rhoC* shRNA-transduced cells expressed lower levels of *klf2* mRNA. This suggests that *rhoA* is involved in the regulation of *klf2* mRNA expression levels.

The Rho effector protein rhophilin 1 is involved in *klf2* mRNA induction. Rho proteins exert their cellular function by binding, in their active GTP-bound state, to Rho effector proteins. To better characterize the RhoA-dependent signaling cascade regulating *klf2* expression, we first investigated the role of the well-characterized effector protein Rho kinase. Treatment of J774A.1 cells with different concentrations of the specific, cell-permeating inhibitor H1152 for different times, however, did not result in *klf2* induction (Fig. 5A). Similar results were obtained with the less specific inhibitor H1077, which also inhibits the Rho effector proteins PRK1, PRK2, and MSK1 (not shown). Thus, Rho kinase seems not to be involved in regulation of *klf2* expression levels.

We next investigated the role of several other Rho effector proteins by shRNA-mediated knockdown. shRNAs specific for *citron*, *rho kinase 2*, *kinectin 1*, and *rhophilin 1* all mediated effective knockdowns of their respective target mRNAs, as measured by real-time reverse transcription-PCR (not shown). Only *rhophilin 1* shRNA-transduced cells, however, induced substantial levels of *klf2* mRNA (Fig. 5B). Importantly, the specificity of the Rhpn-1 small interfering RNA was controlled by extensive manual BLAST searches, which demonstrated no occurrence of the small interfering RNA sequence in any other mRNA transcript (data not shown). Thus, we suggest that a signaling axis via RhoA and rhophilin 1 regulates *klf2* mRNA levels in response to bacterial infection.

DISCUSSION

Recent large-scale gene expression studies identified a number of genes induced by the action of *Yersinia* Yops in macrophages (24, 54) and epithelial cells (8). We have identified the transcription factor *klf2* as one gene with particularly pronounced induction in J774A.1 cells in response to infection with virulent, Yop-translocating yersiniae. Given the immunosuppressive action of *klf2* in a variety of cell types, it seems plausible that *klf2* induction constitutes a novel immunosuppressive strategy of bacteria.

In the case of infection with *Yersinia*, the Rho-inactivating cysteine protease YopT mediates sustained *klf2* expression in host cells. In the absence of YopT, *klf2* is only transiently expressed. *Yersinia* possesses a second Rho-GTPase-inactivating protein, YopE, which acts as a GAP for Rho, Rac, and Cdc42 in vitro (68). However, after infection of living cells with YopE-translocating yersiniae, YopE seems to interact with a much smaller range of Rho proteins: it acts primarily on Rac rather than on Rho or Cdc42 in vivo, and it inhibits Cdc42-mediated Rac activation induced by bradykinin but not direct activation of Rac by sphingosine-1-phosphate (6). Consistently, YopT and YopE have been shown to differently affect the cytoskeleton and phagocytic capacity of DCs (1). Compared to *P. aeruginosa* ExoS, it should be noted that *Yersinia* ExoS contains an additional ADP ribosyltransferase domain inactivating small GTPases (49) and that the GAP domain of

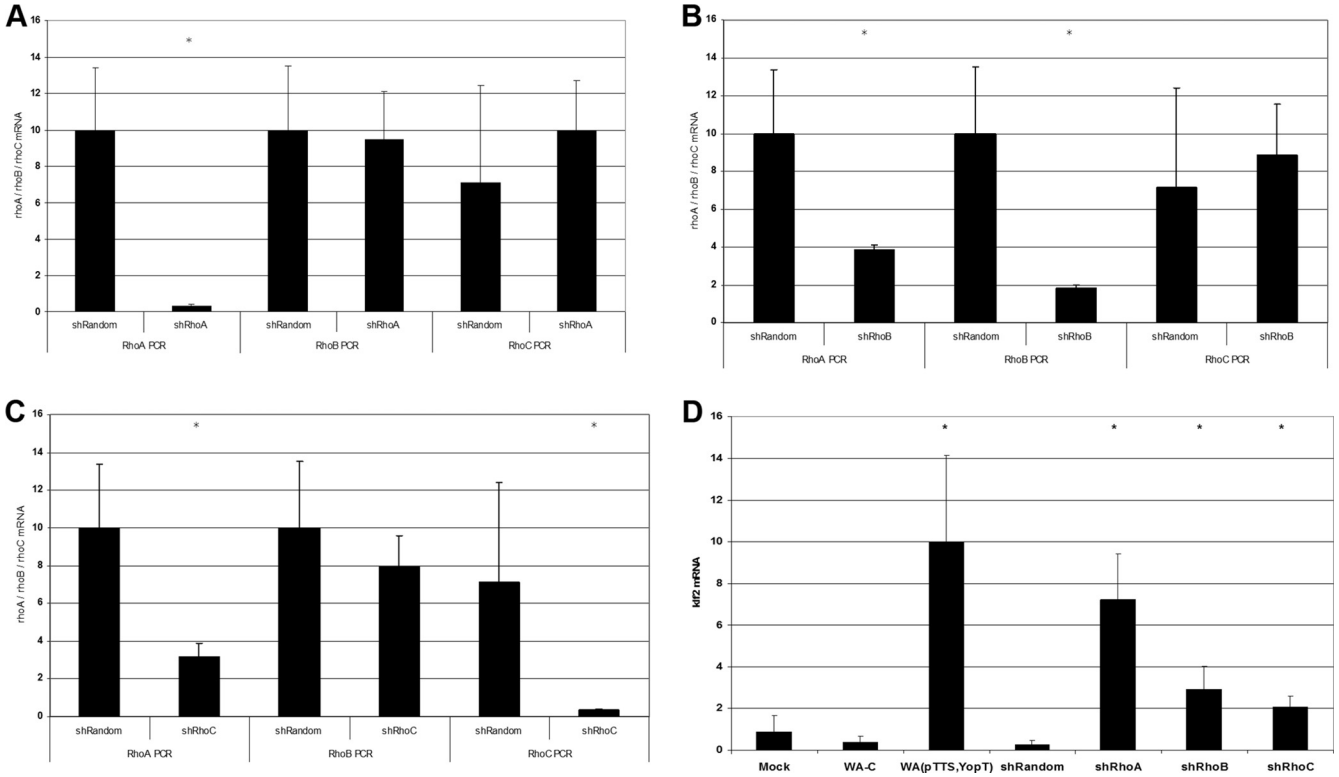

FIG. 4. Effect of shRNA-mediated knockdown of Rho GTPases on *klf2* expression levels. (A) *rhoA*, *rhoB*, and *rhoC* mRNA expression levels in *rhoA* shRNA-transduced cells. (B) *rhoA*, *rhoB*, and *rhoC* mRNA expression levels in *rhoB* shRNA-transduced cells. (C) *rhoA*, *rhoB*, and *rhoC* mRNA expression levels in *rhoC* shRNA-transduced cells. (D) Effect of Rho GTPase knockdown on *klf2* mRNA expression level.

ExoS interacts with a much broader range of host proteins than YopE does (31). Our results are consistent with the targeting of RhoA by ExoS ADP ribosyltransferase, which has not been shown previously. These differences in specificity may explain differences in the *klf2*-inducing capacities of YopT, YopE, and ExoS.

Knowledge about the mechanisms regulating expression of *klf2* is scarce. Tumor necrosis factor receptor-associated factor 2 (TRAF2) and signaling via p38 mitogen-activated protein kinase (MAPK), but not tumor necrosis factor signaling, have been suggested to regulate *klf2* mRNA expression (34). In both human and mouse cells, critical regions within the *klf2* promoter have been identified (27, 57), and heterogeneous nuclear ribonucleoproteins as well as acetyltransferases have been shown to bind to these regions (3). Other results suggest that statin-mediated *klf2* induction in endothelial cells is a result of interference with Rho signaling (58), although the precise mechanism remains obscure. Our results show that a signaling cascade from RhoA via rhophilin 1 regulates *klf2* expression levels. Since it is the inactivation of RhoA and rhophilin 1 which induces *klf2* mRNA expression, this signaling cascade likely suppresses *klf2* expression.

To date, several links between Rho and transcriptional regulation have been described. Rho is required for signaling to Srf by several stimuli (22). However, a direct effector protein of Rho acting on Srf or the *sre* remains to be elucidated. The best-characterized link between Rho and the serum response is via the Srf cofactor Mal. Mal functions as a cytoplasmic sensor for depolymerized actin and is translocated to the nucleus upon actin polymerization, where it serves as a cofactor for Srf (39). Rho has also been shown to indirectly induce the transcriptional activity of NF-κB by phosphorylation of the inhibitor IκBα, enabling active NF-κB to translocate to the nucleus (47). A third link between Rho and transcriptional regulation has been made by demonstrating that Rho is able to stimulate *c-jun* expression via activation of ERK6 (p38γ), a recently identified MAPK (38). However, all of these findings identify Rho as a potent activator, rather than a suppressor, of gene transcription. As far as we are aware, only one other gene besides *klf2* has been described as being suppressed by Rho: expression of the cyclin-dependent kinase inhibitor *cdkn1a* (encoding $p21^{Waf1/Cip1}$) is induced by signaling through activated Ras, and this induction can be inhibited by RhoA by action on the *cdkn1a* promoter (45). Data concerning the rho effector responsible for inhibition of *cdkn1a* expression are conflicting: expression of *cdkn1a* protein could be induced by ROCK inhibition in phorbol myristate acetate-treated human erythromyeloblast D2 cells (33) but not in Ras-V12-transformed Swiss 3T3 cells (52). However, we do not think that any of these signaling pathways is responsible for YopT-mediated regulation of *klf2* expression, for the following reasons: first, we did not find any influence of YopT on expression of *cdkn1a* in J774A.1 cells; second, we could not demonstrate an effect of SRF activity on *klf2* expression levels (data not shown); third, by performing experiments with the actin-depolymerizing agents latrunculin B and cytochalasin D, we could show that

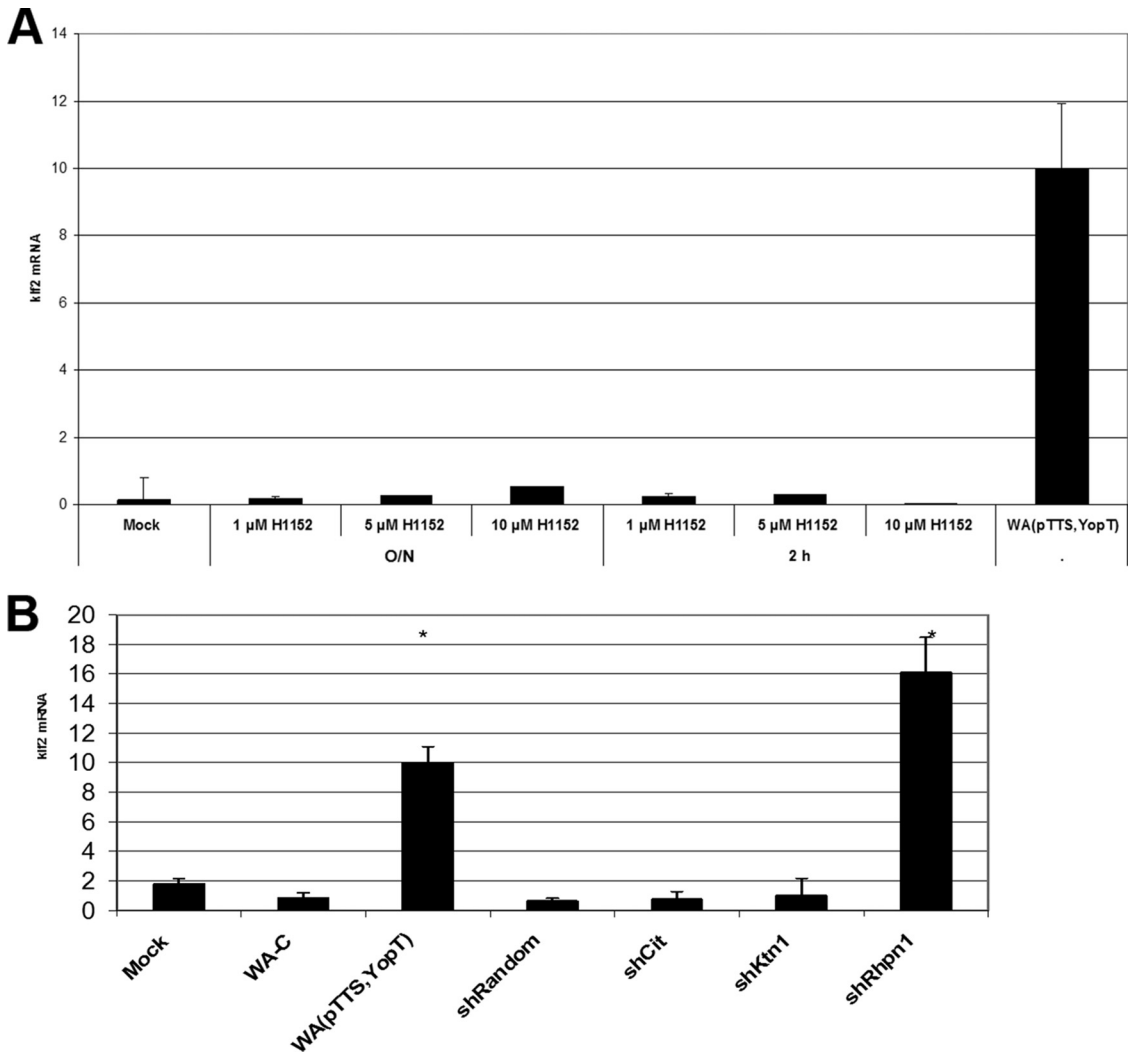

FIG. 5. Impact of Rho effector proteins on *klf2* expression level. (A) *klf2* mRNA levels in J774A.1 cells treated with the ROCK inhibitor H1152 for the indicated times (O/N, overnight) at the indicated concentrations. Similar results were obtained with the inhibitor H1077. (B) Impact of indicated Rho effector protein knockdown by shRNA on *klf2* expression level.

the actin polymerization status is not involved in regulating *klf2* expression (data not shown); and fourth, we found rhophilin 1 rather than ROCK to be involved in *klf2* regulation.

Rhophilin 1 was first described in 1996, when it was detected in a yeast two-hybrid screen with Rho as bait. It interacts strongly with GTP-bound RhoA, less with RhoB, and little with RhoC (71). Rhpn1 is expressed in various tissues (41) and is highly expressed in the mouse testis, where it interacts with ropporin (18). Other putative binding partners have been identified by yeast two-hybrid screens. Among these are Trim37, Krt15, Cnksr1, Efemp2, and Ndp52 (51). Rhpn1 contains several protein-protein interaction motifs, such as HR1, a central BRO1 domain, and a C-terminal PDZ domain (46). The HR1 domain or Rho binding domain was first described as part of protein kinase PRK1, which binds RhoA (15). It is found in a range of signaling proteins, including rhotekin and PRK2, and is required for GTPase binding (50, 71). The exact molecular functions of BRO1 domains are not known. They are required for cargo protein deubiquitination and play a role in endosome metabolism (36). PDZ domains are protein interaction domains that are often found in multidomain scaffolding proteins that organize intracellular signaling at particular subcellular locations (62). Taken together, the presence of different protein interaction motifs suggest that rhophilin 1 may serve as a signaling protein. Thus, the following sequence of events seems likely in YopT-modified regulation of *klf2* expression. YopT cleaves RhoA from its geranylgeranylated membrane anchor, which must not necessarily inactivate RhoA (72) but could also change the subcellular localization of RhoA sufficiently to inhibit effector protein binding. However, the exact subcellular localization of rhophilin 1 in macrophages is unclear, so it remains to be determined whether YopT-mediated RhoA inactivation or compartmentalization is responsible for inhibiting the RhoA-rhophilin 1 interaction. How exactly rhophilin 1 then regulates *klf2* mRNA expression remains to be determined.

We described earlier that *klf2* is also induced transiently ($\leq$1 h after infection) by Yop-devoid yersiniae. Translocation of

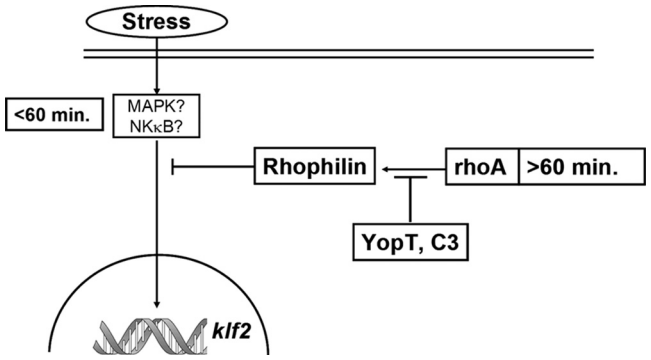

FIG. 6. Proposed model of *klf2* regulation in the context of bacterial infection. Immediately after bacterial contact, host cells induce *klf2* by an as yet uncharacterized signaling cascade; however, MAPK or NF-κB signaling may be involved. Two hours after infection, signaling via RhoA and rhophilin 1 suppresses *klf2* expression. Bacteria mediate long-term expression of *klf2* by suppressing this inhibitory action of RhoA via Rho-inactivating enzymes.

YopT results in a sustained (≥2 h) expression of *klf2* mRNA (24). Together with the data presented here, this suggests the following model of *klf2* regulation in the context of bacterial infection (Fig. 6). Immediately after bacterial contact, host cells induce *klf2* by an as yet uncharacterized signaling cascade, but MAPK or NF-κB signaling may be involved. This early induction of *klf2* may be viewed as a physiological regulatory "loop" to prevent overwhelming inflammatory activation of the host cell. Two hours after infection, signaling via RhoA and rhophilin 1 suppresses *klf2* expression. Bacteria mediate long-term expression of *klf2* by suppressing this inhibitory action of RhoA via Rho-inactivating enzymes. As noted above, this may constitute a novel immunosuppressive strategy. Future studies will identify the relevance of *klf2* induction for infections in vivo as well as the precise role of rhophilin 1 in suppression of transcriptional responses to bacterial infection.

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REFERENCES

1. **Adkins, I., M. Koberle, S. Grobner, E. Bohn, I. B. Autenrieth, and S. Borgmann.** 2007. Yersinia outer proteins E, H, P, and T differentially target the cytoskeleton and inhibit phagocytic capacity of dendritic cells. Int. J. Med. Microbiol. **297:**235–244.
2. **Aepfelbacher, M., C. Trasak, G. Wilharm, A. Wiedemann, K. Trulzsch, K. Krauss, P. Gierschik, and J. Heesemann.** 2003. Characterization of YopT effects on Rho GTPases in Yersinia enterocolitica-infected cells. J. Biol. Chem. **278:**33217–33223.
3. **Ahmad, N., and J. B. Lingrel.** 2005. Kruppel-like factor 2 transcriptional regulation involves heterogeneous nuclear ribonucleoproteins and acetyltransferases. Biochemistry **44:**6276–6285.
4. **Aktories, K., and J. T. Barbieri.** 2005. Bacterial cytotoxins: targeting eukaryotic switches. Nat. Rev. Microbiol. **3:**397–410.
5. **Anderson, K. P., C. B. Kern, S. C. Crable, and J. B. Lingrel.** 1995. Isolation of a gene encoding a functional zinc finger protein homologous to erythroid Kruppel-like factor: identification of a new multigene family. Mol. Cell. Biol. **15:**5957–5965.
6. **Andor, A., K. Trulzsch, M. Essler, A. Roggenkamp, A. Wiedemann, J. Heesemann, and M. Aepfelbacher.** 2001. YopE of Yersinia, a GAP for Rho GTPases, selectively modulates Rac-dependent actin structures in endothelial cells. Cell. Microbiol. **3:**301–310.
7. **Barz, C., T. N. Abahji, K. Trulzsch, and J. Heesemann.** 2000. The Yersinia Ser/Thr protein kinase YpkA/YopO directly interacts with the small GTPases RhoA and Rac-1. FEBS Lett. **482:**139–143.
8. **Bohn, E., S. Muller, J. Lauber, R. Geffers, N. Speer, C. Spieth, J. Krejci, B. Manncke, J. Buer, A. Zell, and I. B. Autenrieth.** 2004. Gene expression patterns of epithelial cells modulated by pathogenicity factors of Yersinia enterocolitica. Cell. Microbiol. **6:**129–141.
9. **Boyd, A. P., N. Grosdent, S. Totemeyer, C. Geuijen, S. Bleves, M. Iriarte, I. Lambermont, J. N. Octave, and G. R. Cornelis.** 2000. Yersinia enterocolitica can deliver Yop proteins into a wide range of cell types: development of a delivery system for heterologous proteins. Eur. J. Cell Biol. **79:**659–671.
10. **Buckley, A. F., C. T. Kuo, and J. M. Leiden.** 2001. Transcription factor LKLF is sufficient to program T cell quiescence via a c-Myc-dependent pathway. Nat. Immunol. **2:**698–704.
11. **Cornelis, G. R.** 2002. The Yersinia Ysc-Yop 'type III' weaponry. Nat. Rev. Mol. Cell Biol. **3:**742–752.
12. **Cox, G. W., B. J. Mathieson, L. Gandino, E. Blasi, D. Radzioch, and L. Varesio.** 1989. Heterogeneity of hematopoietic cells immortalized by v-myc/v-raf recombinant retrovirus infection of bone marrow or fetal liver. J. Natl. Cancer Inst. **81:**1492–1496.
13. **Czech, A., T. Yamaguchi, L. Bader, S. Linder, K. Kaminski, M. Sugai, and M. Aepfelbacher.** 2001. Prevalence of Rho-inactivating epidermal cell differentiation inhibitor toxins in clinical Staphylococcus aureus isolates. J. Infect. Dis. **184:**785–788.
14. **Dekker, R. J., S. van Soest, R. D. Fontijn, S. Salamanca, P. G. de Groot, E. VanBavel, H. Pannekoek, and A. J. Horrevoets.** 2002. Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Kruppel-like factor (KLF2). Blood **100:**1689–1698.
15. **Flynn, P., H. Mellor, R. Palmer, G. Panayotou, and P. J. Parker.** 1998. Multiple interactions of PRK1 with RhoA. Functional assignment of the Hr1 repeat motif. J. Biol. Chem. **273:**2698–2705.
16. **Frank, D. W., G. Nair, and H. P. Schweizer.** 1994. Construction and characterization of chromosomal insertional mutations of the *Pseudomonas aeruginosa* exoenzyme S *trans*-regulatory locus. Infect. Immun. **62:**554–563.
17. **Frankel, G., A. D. Phillips, M. Novakova, H. Field, D. C. Candy, D. B. Schauer, G. Douce, and G. Dougan.** 1996. Intimin from enteropathogenic *Escherichia coli* restores murine virulence to a *Citrobacter rodentium eaeA* mutant: induction of an immunoglobulin A response to intimin and EspB. Infect. Immun. **64:**5315–5325.
18. **Fujita, A., K. Nakamura, T. Kato, N. Watanabe, T. Ishizaki, K. Kimura, A. Mizoguchi, and S. Narumiya.** 2000. Ropporin, a sperm-specific binding protein of rhophilin, that is localized in the fibrous sheath of sperm flagella. J. Cell Sci. **113:**103–112.
19. **Gansheroff, L. J., M. R. Wachtel, and A. D. O'Brien.** 1999. Decreased adherence of enterohemorrhagic *Escherichia coli* to HEp-2 cells in the presence of antibodies that recognize the C-terminal region of intimin. Infect. Immun. **67:**6409–6417.
20. **Gebert, B., W. Fischer, E. Weiss, R. Hoffmann, and R. Haas.** 2003. Helicobacter pylori vacuolating cytotoxin inhibits T lymphocyte activation. Science **301:**1099–1102.
21. **Heesemann, J., and R. Laufs.** 1983. Construction of a mobilizable *Yersinia enterocolitica* virulence plasmid. J. Bacteriol. **155:**761–767.
22. **Hill, C. S., J. Wynne, and R. Treisman.** 1995. The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. Cell **81:**1159–1170.
23. **Hoang, T. T., R. R. Karkhoff-Schweizer, A. J. Kutchma, and H. P. Schweizer.** 1998. A broad-host-range Flp-FRT recombination system for site-specific excision of chromosomally-located DNA sequences: application for isolation of unmarked Pseudomonas aeruginosa mutants. Gene **212:**77–86.
24. **Hoffmann, R., K. Van Erp, K. Trulzsch, and J. Heesemann.** 2004. Transcriptional responses of murine macrophages to infection with Yersinia enterocolitica. Cell. Microbiol. **6:**377–390.
25. **Hoiseth, S. K., and B. A. Stocker.** 1981. Aromatic-dependent Salmonella typhimurium are non-virulent and effective as live vaccines. Nature **291:**238–239.
26. **Hornef, M. W., A. Roggenkamp, A. M. Geiger, M. Hogardt, C. A. Jacobi, and J. Heesemann.** 2000. Triggering the ExoS regulon of Pseudomonas aeruginosa: a GFP-reporter analysis of exoenzyme (Exo) S, ExoT and ExoU synthesis. Microb. Pathog. **29:**329–343.
27. **Huddleson, J. P., S. Srinivasan, N. Ahmad, and J. B. Lingrel.** 2004. Fluid shear stress induces endothelial KLF2 gene expression through a defined promoter region. Biol. Chem. **385:**723–729.
28. **Imanishi, K., K. Yamaguchi, M. Suzuki, S. Honda, N. Yanaihara, and K. Abe.** 1989. Production of transforming growth factor-alpha in human tumour cell lines. Br. J. Cancer **59:**761–765.
29. **Janda, J. M., S. L. Abbott, and M. J. Albert.** 1999. Prototypal diarrheagenic strains of *Hafnia alvei* are actually members of the genus *Escherichia*. J. Clin. Microbiol. **37:**2399–2401.
30. **Kaufman, M. R., J. Jia, L. Zeng, U. Ha, M. Chow, and S. Jin.** 2000. Pseudomonas aeruginosa mediated apoptosis requires the ADP-ribosylating activity of exoS. Microbiology **146:**2531–2541.
31. **Krall, R., J. Sun, K. J. Pederson, and J. T. Barbieri.** 2002. In vivo Rho GTPase-activating protein activity of *Pseudomonas aeruginosa* cytotoxin ExoS. Infect. Immun. **70:**360–367.
32. **Kuo, C. T., M. L. Veselits, K. P. Barton, M. M. Lu, C. Clendenin, and J. M.**

Leiden. 1997. The LKLF transcription factor is required for normal tunica media formation and blood vessel stabilization during murine embryogenesis. Genes Dev. **11:**2996–3006.
33. **Lai, J. M., S. Wu, D. Y. Huang, and Z. F. Chang.** 2002. Cytosolic retention of phosphorylated extracellular signal-regulated kinase and a Rho-associated kinase-mediated signal impair expression of p21(Cip1/Waf1) in phorbol 12-myristate-13-acetate-induced apoptotic cells. Mol. Cell. Biol. **22:**7581–7592.
34. **Lin, Y., J. Ryan, J. Lewis, M. A. Wani, J. B. Lingrel, and Z. G. Liu.** 2003. TRAF2 exerts its antiapoptotic effect by regulating the expression of Kruppel-like factor LKLF. Mol. Cell. Biol. **23:**5849–5856.
35. **Locher, M., B. Lehnert, K. Krauss, J. Heesemann, M. Groll, and G. Wilharm.** 2005. Crystal structure of the Yersinia enterocolitica type III secretion chaperone SycT. J. Biol. Chem. **280:**31149–31155.
36. **Luhtala, N., and G. Odorizzi.** 2004. Bro1 coordinates deubiquitination in the multivesicular body pathway by recruiting Doa4 to endosomes. J. Cell Biol. **166:**717–729.
37. **Lutz, M. B., N. Kukutsch, A. L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler.** 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J. Immunol. Methods **223:**77–92.
38. **Marinissen, M. J., M. Chiariello, and J. S. Gutkind.** 2001. Regulation of gene expression by the small GTPase Rho through the ERK6 (p38 gamma) MAP kinase pathway. Genes Dev. **15:**535–553.
39. **Miralles, F., G. Posern, A. I. Zaromytidou, and R. Treisman.** 2003. Actin dynamics control SRF activity by regulation of its coactivator MAL. Cell **113:**329–342.
40. **Moreilhon, C., D. Gras, C. Hologne, O. Bajolet, F. Cottrez, V. Magnone, M. Merten, H. Groux, E. Puchelle, and P. Barbry.** 2005. Live Staphylococcus aureus and bacterial soluble factors induce different transcriptional responses in human airway cells. Physiol. Genomics **20:**244–255.
41. **Nakamura, K., A. Fujita, T. Murata, G. Watanabe, C. Mori, J. Fujita, N. Watanabe, T. Ishizaki, O. Yoshida, and S. Narumiya.** 1999. Rhophilin, a small GTPase Rho-binding protein, is abundantly expressed in the mouse testis and localized in the principal piece of the sperm tail. FEBS Lett. **445:**9–13.
42. **Navarro, L., A. Koller, R. Nordfelth, H. Wolf-Watz, S. Taylor, and J. E. Dixon.** 2007. Identification of a molecular target for the Yersinia protein kinase A. Mol. Cell **26:**465–477.
43. **O'Grady, E., H. Mulcahy, C. Adams, J. P. Morrissey, and F. O'Gara.** 2007. Manipulation of host Kruppel-like factor (KLF) function by exotoxins from diverse bacterial pathogens. Nat. Rev. Microbiol. **5:**337–341.
44. **O'Grady, E. P., H. Mulcahy, J. O'Callaghan, C. Adams, and F. O'Gara.** 2006. *Pseudomonas aeruginosa* infection of airway epithelial cells modulates expression of Kruppel-like factors 2 and 6 via RsmA-mediated regulation of type III exoenzymes S and Y. Infect. Immun. **74:**5893–5902.
45. **Olson, M. F., H. F. Paterson, and C. J. Marshall.** 1998. Signals from Ras and Rho GTPases interact to regulate expression of p21Waf1/Cip1. Nature **394:**295–299.
46. **Peck, J. W., M. Oberst, K. B. Bouker, E. Bowden, and P. D. Burbelo.** 2002. The RhoA-binding protein, rhophilin-2, regulates actin cytoskeleton organization. J. Biol. Chem. **277:**43924–43932.
47. **Perona, R., S. Montaner, L. Saniger, I. Sanchez-Perez, R. Bravo, and J. C. Lacal.** 1997. Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. Genes Dev. **11:**463–475.
48. **Prentki, P., and H. M. Krisch.** 1984. In vitro insertional mutagenesis with a selectable DNA fragment. Gene **29:**303–313.
49. **Radke, J., K. J. Pederson, and J. T. Barbieri.** 1999. *Pseudomonas aeruginosa* exoenzyme S is a biglutamic acid ADP-ribosyltransferase. Infect. Immun. **67:**1508–1510.
50. **Reid, T., T. Furuyashiki, T. Ishizaki, G. Watanabe, N. Watanabe, K. Fujisawa, N. Morii, P. Madaule, and S. Narumiya.** 1996. Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and rhophilin in the rho-binding domain. J. Biol. Chem. **271:**13556–13560.
51. **Rual, J. F., K. Venkatesan, T. Hao, T. Hirozane-Kishikawa, A. Dricot, N. Li, G. F. Berriz, F. D. Gibbons, M. Dreze, N. Ayivi-Guedehoussou, N. Klitgord, C. Simon, M. Boxem, S. Milstein, J. Rosenberg, D. S. Goldberg, L. V. Zhang, S. L. Wong, G. Franklin, S. Li, J. S. Albala, J. Lim, C. Fraughton, E. Llamosas, S. Cevik, C. Bex, P. Lamesch, R. S. Sikorski, J. Vandenhaute, H. Y. Zoghbi, A. Smolyar, S. Bosak, R. Sequerra, L. Doucette-Stamm, M. E. Cusick, D. E. Hill, F. P. Roth, and M. Vidal.** 2005. Towards a proteome-scale map of the human protein-protein interaction network. Nature **437:**1173–1178.
52. **Sahai, E., M. F. Olson, and C. J. Marshall.** 2001. Cross-talk between Ras and Rho signaling pathways in transformation favours proliferation and increased motility. EMBO J. **20:**755–766.
53. **Sansonetti, P. J., D. J. Kopecko, and S. B. Formal.** 1982. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. Infect. Immun. **35:**852–860.
54. **Sauvonnet, N., B. Pradet-Balade, J. A. Garcia-Sanz, and G. R. Cornelis.** 2002. Regulation of mRNA expression in macrophages after Yersinia enterocolitica infection. Role of different Yop effectors. J. Biol. Chem. **277:**25133–25142.
55. **Schmitt, W., and R. Haas.** 1994. Genetic analysis of the Helicobacter pylori vacuolating cytotoxin: structural similarities with the IgA protease type of exported protein. Mol. Microbiol. **12:**307–319.
56. **Schneider, U., H. U. Schwenk, and G. Bornkamm.** 1977. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int. J. Cancer **19:**621–626.
57. **Schrick, J. J., M. J. Hughes, K. P. Anderson, M. L. Croyle, and J. B. Lingrel.** 1999. Characterization of the lung Kruppel-like transcription factor gene and upstream regulatory elements. Gene **236:**185–195.
58. **Sen-Banerjee, S., S. Mir, Z. Lin, A. Hamik, G. B. Atkins, H. Das, P. Banerjee, A. Kumar, and M. K. Jain.** 2005. Kruppel-like factor 2 as a novel mediator of statin effects in endothelial cells. Circulation **112:**720–726.
59. **SenBanerjee, S., Z. Lin, G. B. Atkins, D. M. Greif, R. M. Rao, A. Kumar, M. W. Feinberg, Z. Chen, D. I. Simon, F. W. Luscinskas, T. M. Michel, M. A. Gimbrone, Jr., G. Garcia-Cardena, and M. K. Jain.** 2004. KLF2 is a novel transcriptional regulator of endothelial proinflammatory activation. J. Exp. Med. **10:**10.
60. **Shao, F., P. M. Merritt, Z. Bao, R. W. Innes, and J. E. Dixon.** 2002. A Yersinia effector and a Pseudomonas avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. Cell **109:**575–588.
61. **Shao, F., P. O. Vacratsis, Z. Bao, K. E. Bowers, C. A. Fierke, and J. E. Dixon.** 2003. Biochemical characterization of the Yersinia YopT protease: cleavage site and recognition elements in Rho GTPases. Proc. Natl. Acad. Sci. USA **100:**904–909.
62. **Sheng, M., and C. Sala.** 2001. PDZ domains and the organization of supramolecular complexes. Annu. Rev. Neurosci. **24:**1–29.
63. **Snellings, N. J., M. Popek, and L. E. Lindler.** 2001. Complete DNA sequence of *Yersinia enterocolitica* serotype 0:8 low-calcium-response plasmid reveals a new virulence plasmid-associated replicon. Infect. Immun. **69:**4627–4638.
64. **Stover, C. K., X. Q. Pham, A. L. Erwin, S. D. Mizoguchi, P. Warrener, M. J. Hickey, F. S. Brinkman, W. O. Hufnagle, D. J. Kowalik, M. Lagrou, R. L. Garber, L. Goltry, E. Tolentino, S. Westbrock-Wadman, Y. Yuan, L. L. Brody, S. N. Coulter, K. R. Folger, A. Kas, K. Larbig, R. Lim, K. Smith, D. Spencer, G. K. Wong, Z. Wu, I. T. Paulsen, J. Reizer, M. H. Saier, R. E. Hancock, S. Lory, and M. V. Olson.** 2000. Complete genome sequence of Pseudomonas aeruginosa PAO1, an opportunistic pathogen. Nature **406:**959–964.
65. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, J. C. Venter, et al.** 1997. The complete genome sequence of the gastric pathogen Helicobacter pylori. Nature **388:**539–547.
66. **Trulzsch, K., A. Roggenkamp, M. Aepfelbacher, G. Wilharm, K. Ruckdeschel, and J. Heesemann.** 2003. Analysis of chaperone-dependent Yop secretion/translocation and effector function using a mini-virulence plasmid of Yersinia enterocolitica. Int. J. Med. Microbiol. **293:**167–177.
67. **Trulzsch, K., T. Sporleder, E. I. Igwe, H. Russmann, and J. Heesemann.** 2004. Contribution of the major secreted Yops of *Yersinia enterocolitica* O:8 to pathogenicity in the mouse infection model. Infect. Immun. **72:**5227–5234.
68. **Von Pawel-Rammingen, U., M. V. Telepnev, G. Schmidt, K. Aktories, H. Wolf-Watz, and R. Rosqvist.** 2000. GAP activity of the Yersinia YopE cytotoxin specifically targets the Rho pathway: a mechanism for disruption of actin microfilament structure. Mol. Microbiol. **36:**737–748.
69. **Wani, M. A., R. T. Means, Jr., and J. B. Lingrel.** 1998. Loss of LKLF function results in embryonic lethality in mice. Transgenic Res. **7:**229–238.
70. **Wani, M. A., S. E. Wert, and J. B. Lingrel.** 1999. Lung Kruppel-like factor, a zinc finger transcription factor, is essential for normal lung development. J. Biol. Chem. **274:**21180–21185.
71. **Watanabe, G., Y. Saito, P. Madaule, T. Ishizaki, K. Fujisawa, N. Morii, H. Mukai, Y. Ono, A. Kakizuka, and S. Narumiya.** 1996. Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. Science **271:**645–648.
72. **Wong, K. W., and R. R. Isberg.** 2005. Yersinia pseudotuberculosis spatially controls activation and misregulation of host cell Rac1. PLoS Pathog. **1:**e16.
73. **Yao, T., J. Mecsas, J. I. Healy, S. Falkow, and Y. Chien.** 1999. Suppression of T and B lymphocyte activation by a Yersinia pseudotuberculosis virulence factor, yopH. J. Exp. Med. **190:**1343–1350.
74. **Zhu, C., T. S. Agin, S. J. Elliott, L. A. Johnson, T. E. Thate, J. B. Kaper, and E. C. Boedeker.** 2001. Complete nucleotide sequence and analysis of the locus of enterocyte effacement from rabbit diarrheagenic *Escherichia coli* RDEC-1. Infect. Immun. **69:**2107–2115.

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