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SET-domain bacterial effectors target heterochromatin protein 1 to activate host rDNA transcription

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Transcription of rRNA genes (rDNAs) in the nucleolus is regulated by epigenetic chromatin modifications including histone H3 lysine (de)methylation. Here we show that LegAS4, a *Legionella pneumophila* type IV secretion system (TFSS) effector, is targeted to specific rDNA chromatin regions in the host nucleolus. LegAS4 promotes rDNA transcription, through its SET-domain (named after *Drosophila* Su(var)3–9, enhancer of zeste [E(z)], and trithorax [trx]) histone lysine methyltransferase (HKMTase) activity. LegAS4's association with rDNA chromatin is mediated by interaction with host HP1α/γ. *L. pneumophila* infection potently activates rDNA transcription in a TFSS-dependent manner. Other bacteria, including *Bordetella bronchiseptica* and *Burkholderia thailandensis*, also harbour nucleolus-localized LegAS4-like HKMTase effectors. The *B. thailandensis* type III effector BtSET promotes H3K4 methylation of rDNA chromatin, contributing to infection-induced rDNA transcription and bacterial intracellular replication. Thus, activation of host rDNA transcription might be a general bacterial virulence strategy.

Keywords: SET domain; nucleolus; rDNA transcription; HP1; *Legionella pneumophila*

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INTRODUCTION

Histone (de)methylation has a critical role in controlling chromatin dynamics and gene transcription [1,2]. Methylation on distinct histone lysine residues, catalysed by a SET domain of ~130 amino acids [3], is established to associate with different transcriptional states [2]; methylation of histone H3 Lys4 (H3K4) generally marks the transcriptionally active state, whereas H3K9 methylation often signals transcriptional repression. For ribosomal RNA gene (rDNA) transcribed in the nucleolus, transcriptionally active rDNAs are hypomethylated and packed into euchromatic structures that feature H3K4 di-/trimethylation (H3K4me2/3) and H4 acetylation [4,5]. In contrast, H3K9me2 and enrichment of heterochromatin protein 1 (HP1) are associated with CpG-hypermethylated transcriptionally silent rDNA. Histone methylation also plays a role in altering the epigenetic state of rDNA chromatin and regulating RNA polymerase I (Pol I)-mediated rDNA transcription [6,7].

Gram-negative *Legionella pneumophila*, the causative agent of Legionnaires' disease, infects and replicates within the alveolar macrophages [8]. Critical to *L. pneumophila* pathogenesis is a Dot/Icm TFSS that translocates more than 250 effector proteins into host cells to subvert various host functions [9,10]. In this study, we identify LegAS4, a *L. pneumophila* TFSS effector that was localized in the host nucleolus and promoted rDNA transcription. LegAS4 contained an active SET domain that methylated H3. We identify HP 1α/γ as host binding partners of LegAS4, which mediated its association with rDNA chromatin. Other bacterial pathogens, including *Bordetella bronchiseptica* and *Burkholderia thailandensis*, also harbour LegAS4-like HKMTase effectors targeted to the host nucleolus. The *B. thailandensis* type III effector BtSET promoted H3K4 methylation of rDNA chromatin and contributed to infection-induced rDNA transcription. We propose that activation of rDNA transcription might be a general virulence strategy employed by bacterial pathogens for intracellular survival.

RESULTS AND DISCUSSION

LegAS4 is a nucleolar SET-domain effector

Previous bioinformatics efforts have identified LegAS4 as a putative secreted effector owing to the presence of a eukaryotic-specific ankyrin repeat domain [11–13]. The amino terminus of LegAS4 also contained a SET domain and tandem nuclear localization signals (NLS) (Fig 1A). LegAS4 was efficiently translocated from *L. pneumophila* into host macrophages (supplementary Fig S1A,B online). When ectopically expressed in 293T cells, LegAS4 was localized exclusively in the nuclei (Fig 1B). Notably, LegAS4 was concentrated in a discrete nuclear area that resisted staining by 4′,6-diamidino-2-phenylindole.

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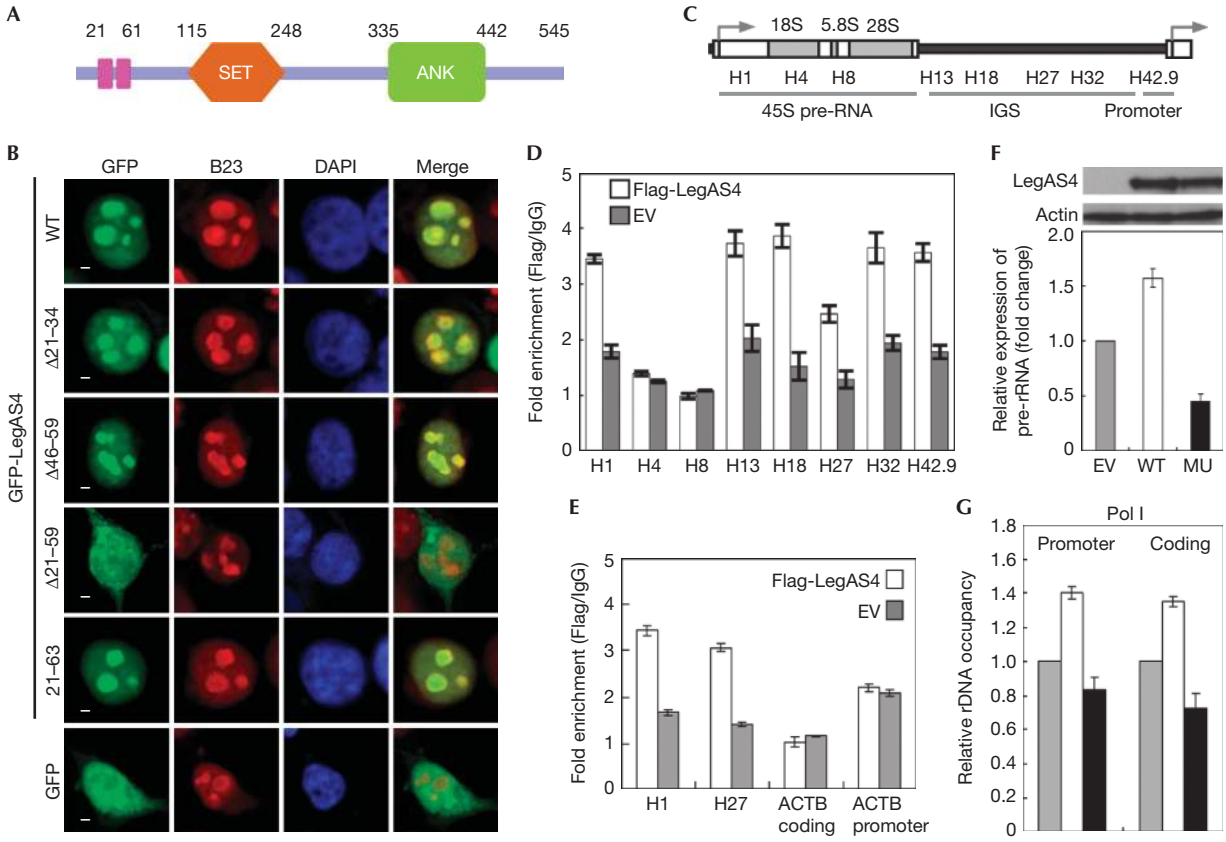

Fig 1 | LegAS4 is a nucleolar protein and activates host rDNA transcription. (**A**) Diagram of LegAS4 domain structure. Pink, the two nuclear localization sequences. (**B**) Nucleolar localization of GFP-LegAS4 in HeLa cells. B23 antibody and 4′,6-diamidino-2-phenylindole stains the nucleolus and DNA, respectively. Scale bar, 2 μm. (**C**) Schematic drawing of a single human rDNA repeat. (**D,E**) ChIP assay of LegAS4 binding to rDNA locus. 293T cells were transfected with an EV or Flag-LegAS4. Quantification of DNA binding was determined by qPCR with primer sets targeting indicated rDNA regions (**D**) or β-actin (ACTB) coding/promoter regions (**E**). Error bars indicate the s.d. of three experiments. (**F**) LegAS4 stimulates rDNA transcription. GFP-LegAS4 (WT or the Y172A/R191D MU) was expressed in 293T cells. Shown are qRT–PCR measurements of the relative pre-rRNA levels normalized to those of β-actin. The western blot shows the expression of GFP-LegAS4. Error bars indicate s.d. of three experiments. (**G**) LegAS4 increases Pol I occupancy at rDNA locus. 293T cells were transfected with GFP-LegAS4 (white bars) or the Y172A/R191D mutant (black bars) or an empty vector control (grey bars). Shown are ChIP analyses of the relative occupancy of Pol I at the rDNA promoter and the 18S rRNA coding region. Error bars indicate s.d. of three experiments. ANK, ankyrin; ChIP, chromatin immunoprecipitation; DAPI, 4′,6-diamidino-2-phenylindole; EV, empty vector; GFP, green fluorescent protein; IgG, immunoglobulin G; IGS, intergenic spacer; MU, mutant; qRT–PCR, quantitative real-time polymerase chain reaction; rRNA, ribosomal RNA.

The nuclear distribution pattern of LegAS4 matched that of B23, a classical nucleolar marker (Fig 1B). Deletion of the SET or ankyrin repeat domain did not alter the nucleolar localization of LegAS4 (supplementary Fig S1C online). Removing either of the tandem NLS sequences also had no effects on LegAS4 localization (Fig 1B and supplementary Fig S1D online), but deletion of both NLS completely disrupted its nucleolar/nuclear localization (Fig 1B). The tandem NLS alone were sufficient to drive the green fluorescent protein fusion protein to the nucleolus (Fig 1B). These establish the nucleolar localization of LegAS4 in eukaryotic cells.

LegAS4 activates rDNA transcription

The nucleolar localization of LegAS4 suggested a function in regulating rDNA transcription. To this end, chromatin immunoprecipitation was performed to investigate the possible association of LegAS4 with the rDNA (Fig 1C). Quantitative real-time polymerase chain reaction (qRT–PCR) analysis showed that LegAS4 did bind to the rDNA locus, mainly in the promoter and the intergenic spacer regions (Fig 1D). No enrichment of pre-rDNA coding region was observed. The specific binding of LegAS4 to the rDNA was also supported by the lack of LegAS4 enrichment on the promoter and coding regions of β-actin (Fig 1E). Expression of LegAS4 in 293T cells, but not its HKMTase-deficient mutant (Y172A/R191D), resulted in a significant increase of 45S pre-ribosomal RNA synthesis (Fig 1F). The Y172A/R191D mutant exhibited a dominant-negative effect (see below). Furthermore, LegAS4 expression promoted Pol I occupancy at both rDNA promoter and coding regions, while the Y172A/R191D mutant showed a slightly negative effect (Fig 1G). Thus, nucleolus-localized LegAS4 is associated with rDNA chromatin and can stimulate rDNA transcription.

The SET domain of LegAS4 is catalytically active

The SET domain of LegAS4 shares the highest sequence identity of ~35% to that of NSD2/3, and therefore is more closely related to the SET2 family (supplementary Fig S2A online). Residues critical for HTMase activity, including the GXG (X, any residue) binding site for *S*-adenosyl-L-methionine (SAM), the YXG catalytic motif and the characteristic pseudoknot structure, are highly conserved in LegAS4. Bacterially purified glutathione *S*-transferase (GST)-LegAS4 efficiently and specifically transferred ^{3}H-methyl from SAM onto recombinant histone H3 as well as H3 in core histone (supplementary Fig S2B online). Aspartate substitution for Arg-191, a conserved SAM-binding residue, markedly decreased the HMTase activity of GST-LegAS4 (supplementary Fig S2C online). Thus, LegAS4 is a *bona fide* SET-domain H3KMTase.

Using a panel of 12 methylation-specific H3 antibodies, recombinant LegAS4, used at a minimal amount, catalysed H3K4me2 and to a much less extent H3K9me3 on core histone substrate, but showed no effects on the other 10 H3 methylation modifications (supplementary Fig S2D,E online). However, expression of LegAS4 in 293T cells did not induce significant changes in H3 methylation on all four lysine sites in the total histone pool (not shown). Given that LegAS4 was expressed restrictedly in the nucleolus, histone methylation at the rDNA was then monitored. LegAS4 expression resulted in an increased level of H3K4me2 at the rDNA promoter, but not at the coding region (supplementary Fig S2F online). LegAS4 did not stimulate H3K4 mono-/trimethylation and H3K9 mono-/di-methylation. H3K9me3 level was also not affected, consistent with the fact that H3K9me2/3 functions in heterochromatin formation and rDNA silencing [5,14]. The Y172A/R191D mutant of LegAS4 exhibited a dominant-negative effect of decreasing H3K4me2 (supplementary Fig S2F online), agreeing with its inhibitory function on rDNA transcription (Fig 1F).

LegAS4 specifically binds to HP1α/γ proteins

A yeast two-hybrid screen using LegAS4 ΔSET as the bait identified two clones encoding HP1α and HP1γ from a HeLa cDNA library. The HP1 family contains a third isoform HP1β, and the three isoforms function redundantly under certain situations [15,16]. LegAS4 interacted robustly with HP1α and HP1γ, but extremely weakly with HP1β (Fig 2A). LegAS4 did not interact with B23. Flag-LegAS4 could efficiently pull down HP1α and HP1γ, but not HP1β, from 293T cells (Fig 2B). HP1α localized mainly at chromocentres in the interphase nuclei of 3T3 fibroblasts [17], but was relocalized to the nucleolus when LegAS4 was present (supplementary Fig S3 online).

HP1 contains an N-terminal chromodomain and a carboxy terminal chromo shadow domain (CSD) (Fig 2C), which mediate binding to methylated histone H3K9 and dimerization/interaction with other HP1-binding proteins, respectively. The CSD domain, but not the chromodomain, was sufficient for a robust binding to LegAS4 (Fig 2D). Two mutations within the CSD domain (I165E and W174A) (Fig 2C), known to disrupt HP1 dimerization and interaction with HP1-binding proteins [18], also abolished HP1α interaction with LegAS4 (Fig 2E). HP1-interacting proteins usually harbour a consensus sequence of PXVXL [X, M/L/V] [18,19], but no such motif could be identified in LegAS4, suggesting a distinct HP1-binding mode. Extensive truncation analyses revealed that the nucleolar localization sequence (NoLS)-containing region of ~40 residues in LegAS4 was required and sufficient for HP1α binding (Fig 2F).

HP1 is required for LegAS4's association with rDNA

RNAi knockdown of HP1α or HP1γ alone, or both simultaneously, did not alter the nucleolar localization of LegAS4 in 293T cells (supplementary Fig S4A,B online). Purified HP1α did not affect the HTMase activity of LegAS4 (supplementary Fig S4C online). Depletion of HP1α or HP1γ alone had no evident effects on the enrichment of rDNA in Flag-LegAS4 immunoprecipitates (supplementary Fig S4D,E online), but simultaneous knockdown of both HP1α and HP1γ resulted in a significantly decreased rDNA precipitation by Flag-LegAS4 (Fig 2G). The defective recruitment of LegAS4 to rDNA chromatin in HP1 knockdown cells was observed only when primers targeting the LegAS4-associated rDNA region were used in the chromatin immunoprecipitation–quantitative polymerase chain reaction assay (Figs 1D and 2G). HP1 selectively binds to H3K9me3 and is enriched in transcriptionally silent rDNA chromatin [5,7]. Thus, the high-affinity HP1 binding is responsible for specific recruitment of LegAS4 to the transcriptionally silent rDNA chromatin region.

LegAS4-like rDNA-associated SET-domain effectors

PSI-BLAST search identified a large family of LegAS4-like SET-domain proteins from various bacteria, including those infecting plants (Fig 3A). Among them, NP_891504 from *B. bronchiseptica* RB50 (named as BbSET) and YP_443833 from *B. thailandensis* E264 (named as BtSET) exhibited LegAS4-like nucleolar localization in HeLa cells (Fig 3B). Ectopically expressed Flag-BtSET was enriched in the rDNA promoter and transcription initiation regions, but not in the coding and intergenic spacer regions (Fig 3C). Recombinant GST-BtSET, but not the catalytic residues mutant (Y33A/R78D), specifically methylated H3 in core histone (Fig 3D). BtSET preferred to target H3K4 for mono-/di-methylation *in vitro* (Fig 3E,F). Thus, BtSET functions similarly as LegAS4 to promote H3K4 methylation at the rDNA, probably activating rDNA transcription.

Legionella activates rDNA transcription

The above analyses raised an illuminating idea that bacterial pathogens might epigenetically modulate host rDNA transcription. To this end, *L. pneumophila* infections of *Acanthamoeba castellanii*, mouse bone marrow-derived macrophages as well as human U937 monocytes were carried out. Wild-type *L. pneumophila*, but not the *dotA* mutant, stimulated rDNA transcription by several folds in all the three cell types (supplementary Fig S5A online). Addition of actinomycin D (ActD) at the concentration that only blocks Pol I activity reversed Dot/Icm-stimulated rDNA transcription (supplementary Fig S5B online). LegAS4, translocated through the Dot/Icm system, was found to colocalize with Pol I in the nucleolus of infected host cells (supplementary Fig S5C online). However, deletion of LegAS4 from the bacteria did not affect rDNA transcription in U937 cells and *A. castellanii* (supplementary Fig S5D online). RNA-Seq transcriptional profiling revealed no significant difference between U937 cells infected with wild-type *L. pneumophila* and those infected with the LegAS4 deletion strain (supplementary Fig S6 and supplementary Data set S1 online). This is not unexpected given that the *L. pneumophila* Dot/Icm system

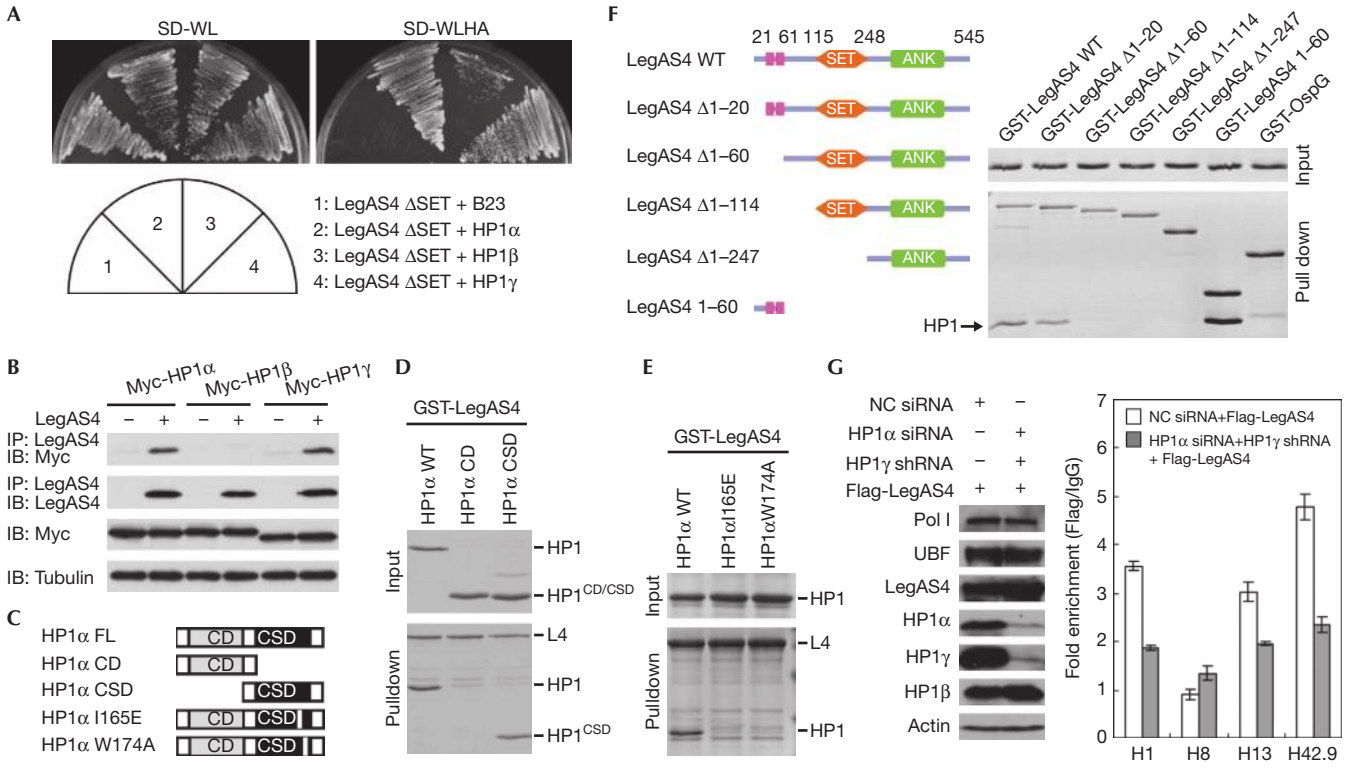

Fig 2 | HP1 is required for LegAS4 association with the rDNA. (**A**) Identification of HP1α/γ as the binding factors of LegAS4 by yeast two-hybrid screen. (**B**) Co-immunoprecipitation of LegAS4 with HP1 in HEK293T cells. (**C**) Domain structures of HP1α and its mutants subjected to LegAS4 binding assay in (**D**,**E**). (**D**,**E**) *In vitro* pulldown assay of LegAS4 binding to various HP1α mutants. (**F**) Mapping the HP1 binding region in LegAS4 by *in vitro* pulldown assay. GST-OspG protein (a type III effector from *Shigella flexneri*) was included as a negative control. (**G**) Effects of HP1α and HP1γ double knockdown on LegAS4 binding onto the rDNA. HP1α-targeting siRNA was transfected into the HP1γ shRNA stable knockdown 293T cells. Knockdown cells were transfected with Flag-LegAS4 for another 48 h followed by anti-Flag ChIP assay using primer sets targeting indicated rDNA regions. Error bars indicate s.d. of three experiments. ANK, ankyrin; CD, chromodomain; CSD, chromo shadow domain; ChIP, chromatin immunoprecipitation; GST, glutathione *S*-transferase; HEK, human embryonic kidney; HP1, heterochromatin protein 1; $HP1^{CD}$, HP1α CD domain; $HP1^{CSD}$, HP1α CSD domain; L4, GST-LegAS4; IgG, immunoglobulin G; shRNA, short hairpin RNA; siRNA, short interfering RNA; UBF, upstream binding factor.

delivers more than 250 effectors into host cells and functional redundancy is commonly seen. Notwithstanding, these data do establish that intracellular *L. pneumophila* relies on its TFSS system to manipulate host rDNA transcription.

BtSET activates host rDNA transcription

The function of other SET-domain effectors was then examined. Infection of 293T cells with wild-type *B. thailandensis* E264 also stimulated rDNA transcription by nearly fivefolds (Fig 4A). In contrast, the type III-deficient Δ*bipB* mutant did not show such effect. BtSET was found to be efficiently translocated into host cells by the *B. thailandensis* type III secretion system (Fig 4B). Genetic ablation of the BtSET-encoding gene largely blocked infection-induced rDNA transcription, which was fully restored by a BtSET-expressing plasmid (Fig 4A).

BtSET contributes to bacterial intracellular replication

Similarly to that observed with *L. pneumophila*, inhibition of Pol I activity by ActD completely blocked *B. thailandensis*-activated rDNA transcription (Fig 4A). When HeLa cells were infected with *B. thailandensis* at a low multiplicity of infection (MOI) in the presence of ActD, bacterial intracellular replication was completely suppressed (Fig 4C). ActD treatment did not affect bacterial growth in the absence of infection (Fig 4C). Consistently, deletion of BtSET inhibited intracellular replication of the bacteria (Fig 4D). Moreover, short interfering RNA knockdown of TAF48 (Fig 4E), a major component of the SL1 complex responsible for recruiting Pol I to the transcription start site [20], not only resulted in an inhibition of pre-rRNA synthesis (Fig 4F), but also significantly blocked *B. thailandensis* intracellular replication (Fig 4G). Thus, activation of rDNA transcription is important for facilitating *B. thailandensis* multiplication in host cells.

Bacterial remodelling of host chromatin structure is an emerging concept [21]. The LegAS4 family of SET-domain bacterial effectors is unique in that it features nucleolar localization, rDNA association and activation of host rDNA transcription. These SET-domain bacterial proteins belong to an emerging class of bacterial factors targeting the nucleus, named as 'nucleomodulins' [22]. The wide presence of LegAS4-like

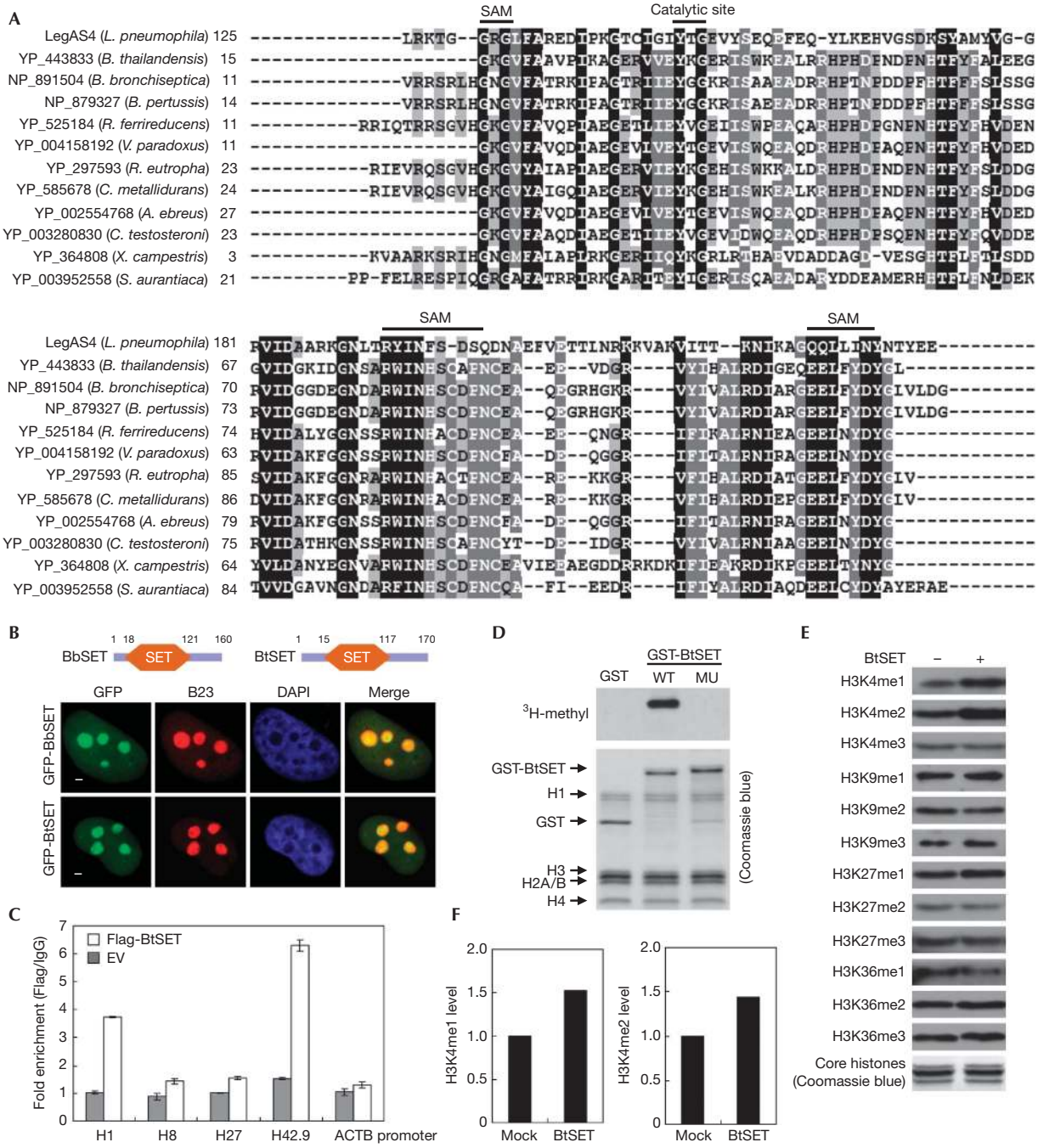

Fig 3 | LegAS4 defines a family of rDNA-associated SET-domain bacterial effectors. (**A**) Multiple sequence alignments of the LegAS4 family of bacterial SET-domain proteins. The LegAS4 family contains YP_443833 from *B. thailandensis* E264 (renamed as BtSET), NP_879327 from *Bordetella pertussis* Tohama I, NP_891504 from *B. bronchiseptica* RB50 (renamed as BbSET), YP_525184 from *Rhodoferax ferrireducens* T118, YP_004158192 from *Variovorax paradoxus* EPS, YP_297593 from *Ralstonia eutropha* JMP134, YP_585678 from *Cupriavidus metallidurans* CH34, YP_002554768 from *Acidovorax ebreus* TPSY, YP_003280830 from *Comamonas testosteroni* CNB-2, YP_364808 from *Xanthomonas campestris* and YP_003952558 *Stigmatella aurantiaca* DW4 3-1. (**B**) Domain diagram and nucleolar localization of BbSET and BtSET. HeLa cells were transfected with GFP-BbSET or BtSET. Scale bar, 2 μm. (**C**) BtSET specifically binds to the rDNA locus. The experiments were performed and data are presented similarly as those in Fig 1D. Error bars indicate s.d. of three experiments. (**D**) *In vitro* HKMTase assay of BtSET using calf thymus-derived core histone substrate. MU, the Y33A/R78D mutant of purified GST-BtSET. (**E,F**) BtSET prefers to catalyse H3K4me1 and H3K4me2 *in vitro*. Immunoblotting of the reaction mixtures using indicated methylation site-specific antibodies is shown in (**E**). Quantification of H3K4me1 and H3K4me2 is shown in **F** as the average of two experiments (values from mock-treated histone were arbitrarily set as 1). GFP, green fluorescent protein; GST, glutathione *S*-transferase; HKMTase, histone lysine methyltransferase; MU, mutant.

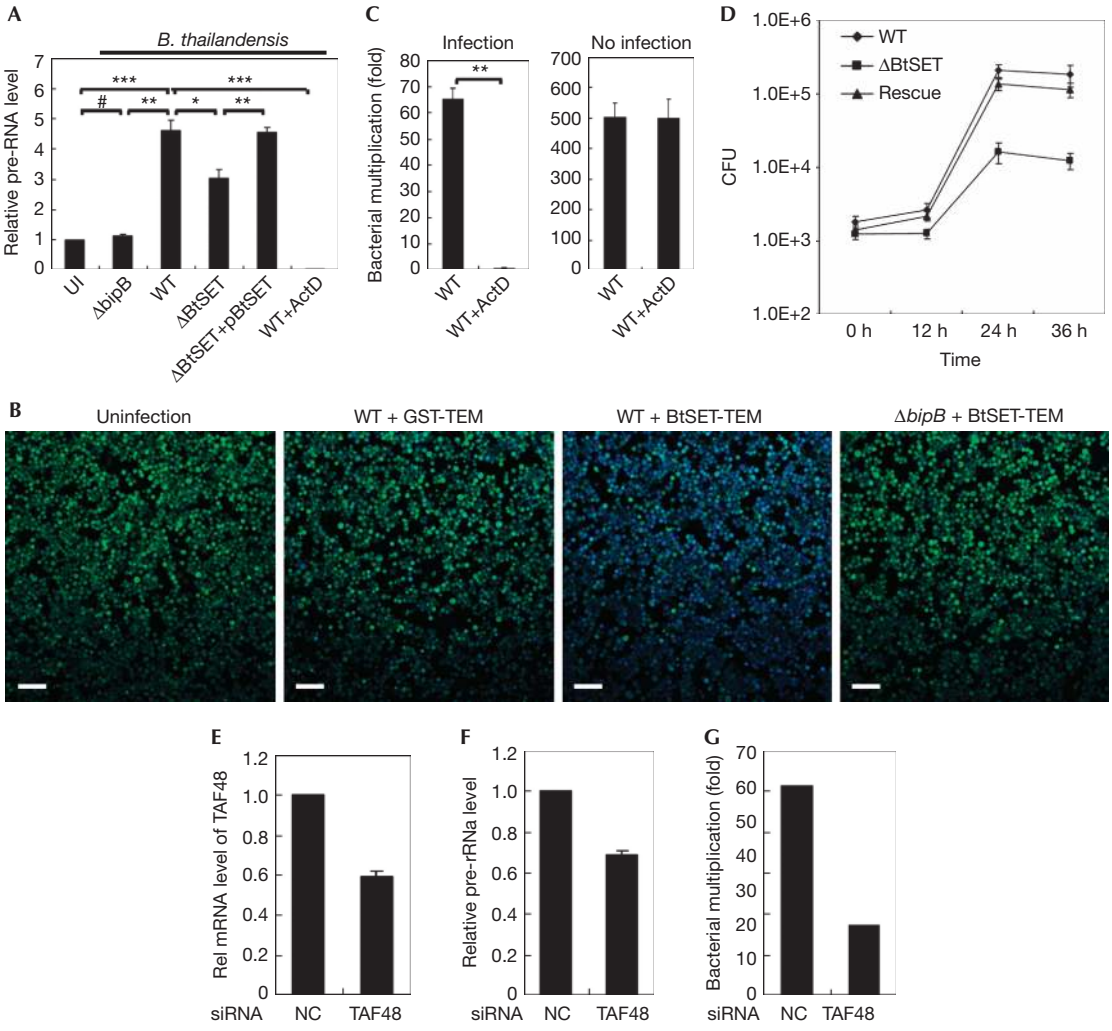

Fig 4 | BtSET activates host rDNA transcription and contributes of *Burkholderia* intracellular replication. (**A**) qRT–PCR assay of BtSET-induced rDNA transcription in 293T cells infected with indicated *B. thailandensis* E264 strains (MOI, 20). Actinomycin D (ActD, 40 ng/ml) is used to block Pol I activity. Error bars indicate s.d. of three experiments. Statistical analysis was done using the Student's *t*-test. $^{*}P<0.5$, $^{**}P<0.05$, $^{***}P<0.001$, $^{\#}P>0.5$. (**B**) BtSET is translocated into host cells through the *B. thailandensis* type III secretion system. U937 cells were infected with *B. thailandensis* or the Δ*bipB* mutant strain harbouring BtSET-TEM or GST-TEM β-lactamase expression plasmids. Scale bar, 100 μm. (**C**) Effects of inhibiting rDNA transcription on *B. thailandensis* intracellular replication. *Left*, *B. thailandensis* infection of HeLa cells (MOI, 1) in the presence of actinomycin D (40 ng/ml) (Student's *t*-test, $^{**}P<0.005$). *Right*, *B. thailandensis* E264 strain was cultured in the DMEM medium in the presence of 40 ng/ml ActD and fold increase of the number of bacteria following replication is shown (Student's *t*-test, $P>0.05$). Error bars indicate s.d. of three experiments. (**D**) Deletion of BtSET inhibits *B. thailandensis* intracellular growth. HeLa cells were infected with indicated *B. thailandensis* strains (MOI, 1). Error bars indicate s.d. of three experiments. (**E–G**) Inhibition of pre-rRNA synthesis suppresses *B. thailandensis* intracellular growth. HeLa cells were transfected with a non-targeting control siRNA (NC) or siRNA-targeting hTAF48. Shown in **E** and **F** are levels of hTAF48 mRNA (knockdown efficiency) and pre-rRNA synthesis, respectively. (Error bars indicate s.d. of three experiments.) In **G**, knockdown cells were infected with *B. thailandensis* (MOI, 1), and bacterial intracellular replication is shown as the average of two experiments. ActD, Actinomycin D; DMEM, Dulbecco's modified Eagle's medium; GST, glutathione *S*-transferase; MOI, multiplicity of infection; mRNA, messenger RNA; NC, non-targeting control; qRT–PCR, quantitative real-time polymerase chain reaction; rRNA, ribosomal RNA; siRNA, short interfering RNA; UI, uninfected.

effectors suggests that activation of host rDNA transcription might be a common virulence strategy for diverse bacterial pathogens. Stimulation of rDNA transcription might contribute to bacterial replication in two flavours. The enforced higher proliferation potential of infected cells, resulting from activation of rDNA transcription, could provide a better niche for bacterial replication. On the other hand, intracellular bacteria could exploit host ribosome activity for its own survival advantages. It is worth mentioning that *Legionella*-containing vacuoles are studded with increasing numbers of ribosomes during the first 8 h following internalization, after which the bacteria start to replicate in the vacuole [10].

While this manuscript was being considered for publication, Rolando *et al* [23] reported that the LegAS4-equivalent effector from *L. pneumophila* strain Paris targets H3K14 for trimethylation to repress expression of host genes involved in innate immune defence. The Rolando study, different from ours, uses recombinant H3 or the octomer as the substrate for site identification and examines the general nuclei function of the LegAS4. Despite these differences, it will be interesting in future studies to investigate whether LegAS4-catalysed H4K14 trimethylation also is important for its nucleolus function and activation of rDNA transcription.

METHODS

Bacteria strains and infection. *L. pneumophila* strains Lp02 (*thyA*, *hsdR* and *rpsL*) and Lp03 (Lp02 *dotA*) were cultured on buffered charcoal yeast extract agar supplemented with 0.1 mg/ml thymidine (BCYET). For assay effector translocation, single colonies of fresh Lp02 or Lp03 strain transformed with TEM1-LegAS4 or TEM1-GST expression plasmid were streaked onto BCYE plates 2 days before infection. Twelve hours before infection, 1×10^5 phorbol myristate acetate-differentiated U937 cells were plated in 24-well format, and cultured in the presence of 0.1 mg/ml thymidine-HCl with no antibiotics. Bacteria were scraped, diluted in sterile water and added to cells at an MOI of 10. Cells were centrifuged immediately at 250*g* for 10 min at 25 °C to enhance bacterial adherence and internalization. Infection was carried out at 37 °C in the presence of 5% CO_2 for 2 h. Cells were then washed three times with PBS and cultured for another hour in the presence of 1 μM CCF2-AM dye. Effector translocation was examined on a confocal fluorescence microscope by measuring blue fluorescence (~450 nm) and green fluorescence emission (~530 nm) on excitation at 405 nm. Adenylate cyclase reporter assay was performed by following a previously established protocol [24]. For assay rDNA transcription, near-confluent cultures of *A. castellanii* were seeded into 24-well plates and incubated overnight in peptone yeast glucose medium. Cells were then washed and equilibrated with *A. castellanii* buffer [25] at 37 °C for 1 h before infection at an MOI of 20. The infection was allowed to proceed for 8 h before extracting the total RNA. For assay activation of rDNA transcription in macrophages, A/J bone marrow-derived macrophages were infected at an MOI of 50 for 6 h and phorbol myristate acetate-differentiated U937 cells were infected at an MOI of 10 for 10 h.

***In vitro* HKMTase assay.** HKMTase assays were carried out in a 20-μl reaction buffered with 50 mM Tris pH 8.0, 20 mM KCl, 250 mM sucrose, 10 mM $MgCl_2$ and 10 mM β-mercaptoethanol. For each reaction, 1 μg of recombinant enzyme was reacted with calf thymus core histones or recombinant H3 in the presence of 1 μCi of *S*-[methyl-^{3}H]-SAM (PerkinElmer) for 1 h at 37 °C. The reaction mixtures were then boiled for 5 min and loaded onto 15% polyacrylamide gel electrophoresis gels. Gels were stained with Coomassie blue to visualize protein loading, and incorporation of ^{3}H was detected by autoradiography.

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

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Author contributions: T.L. designed and performed the large majority of experiments, analysed the data and wrote the manuscript; Q.L., G.W., H.X. and J.G. provided technical assistance; H.H. and T.C. helped with RNA-Seq experiments; B.K. and F.S. provided research and technical support, and F.S. also helped with data analysis and organization of the manuscript.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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