Supplemental information

Context-dependent action of Scc4 reinforces control of the type III secretion system

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Supplemental Table S1 and S2.

Supplemental Figures S1-4.

Supplemental Table S1. Strains, cell lines and plasmids used in this study

	Relevant Details	Reference/Source
Strains		
C. trachomatis		
L2/25667R	Plasmid-free strain	Gong *et al.* (1)
L2/Scc4	L2/25667R transformed with a plasmid containing P_{tet}-directed synthesis of Scc4	This study
L2/vector	L2/25667R transformed with pBOMBP3	Cong *et al.*, (2)
E. coli		
DH5α	Host cells for cloning	Invitrogen
DH5-α F'I^q	C2992, Host cell for cloning	New England Biolabs
BL21(DE3)	Host cell for P_{T7}-directed protein expression	Novogen
GS345	*E. coli* K12 MG1655 wild type σ^{70}	provided by Ann Hochschild
GS235	*E. coli* K12 MG1655 mutant σ^{70}(D581G*)*	as above
GS347	*E. coli* K12 MG1655 mutant σ^{70}(A544I/D581G)	as above
FW102 O_L2-62	*E. coli* reporter strain for B2H assay	Rao *et al.* (3)
ER2925	methylation deficient *E. coli* strain	New England Biolabs
Y. pestis		
YP769	*Y. pestis* strain carrying a plasmid, pCD1-Δ1234, that is deleted for all six effector Yops and their chaperones.	Schesser Bartra *et al*, (4)
YP1283	*Y. pestis* strain carrying a pCD1 plasmid but is deleted for the *yopE* gene (and a *dhfr* gene is inserted)	Schesser Bartra *et al* (4)
Cell line		
L929	Mmouse fibroblast cell	ATCC CCL-1
HeLa 229	Human cervical cancer cell line	ATCC CCL-2.1
Plasmids		
pCDFScc4	$P_{T7/lacUV5}$-directed synthesis of the Scc4 fused to an N-terminal His6-tag	Shen *et al.*, 2015 (5)
pXDCM	P_{T7}-directed synthesis of the Scc4	Rao *et al.*, (3)
pLN-σ^{66R24}	P_{T7}-directed synthesis of *N*-terminal His6-tagged *E. coli* σ^{70} regions 1 (amino acid residues 1-372) fused to regions 2-4 of *C. trachomatis* σ^{66} (amino acid residues 315–571).	Cong *et al.* (2)
pBAD66	P_{ara}-directed synthesis of chlamydial σ^{66} fused to an *N*-terminal His6-tag	Rao *et al.*, (3)
pIA545	P_{trc}-dircted synthesis of *E. coli* β subunit of RNAP fused to an *N*-terminal His6- tag	Artsimovitch *et al.*, (6)
pRV$_{hctB}$	Promoter reporter containing P_{hctB}*::lacZ*;	Hua *et al.* (7)
pRV$_{ompA}$	Promoter reporter containing P_{ompA}*::lacZ*	This study
pQF50KgroE	Promoter reporter containing $P_{E.\ coli\ groE}$::lacZ	Wang *et al.* (8)
pBAD24-CopN	P_{araB}-directed synthesis of *C. trachomatis* CopN	This study
pBAD24-CopNScc1	P_{araB}-directed synthesis of *C. trachomatis* CopN & Scc1	This study
pACλcI-β-flap	P_{lacUV5}-directed synthesis of the λCI protein fused to β-flap of *C. trachomatis*	Rao *et al.*, (3)
pACScc1	P_{lacUV5}-directed synthesis of the λCI protein fused to full length *C. trachomatis* Scc1	Shen *et al.* (5),
pACScc4	P_{lacUV5}-directed synthesis of the λCI protein fused to *C. trachomatis* Scc4	Shen *et al.* (5)
pRB-sycN	P_{lacUV5}/P_{lpp}-directed synthesis of the αNTD of *E. coli* RNAP fused to *Yersinia* SycN	This study
pBR-yscB	P_{lacUV5}/P_{lpp}-directed synthesis of the αNTD fused to *Yersinia* YscB	This study

Table S2. Table S2. Primers used for PCR, qPCR or RT-qPCR

Primer	Sequence (5'–3')	Purpose
*cop*N-rtF	CGCTTCGCTCCTTATATTTC	RT-qPCR
*cop*N-rtR	CCATAACAGCGGTTTTCTCT	
tuf-rtF	GTAACTCTGCCTGAGGGAATTGA	qPCR
tuf-rtR	CACGAATCGCAAATCTCATACCT	RT-qPCR
omcB-rtF	GTTTGCGTTGCCCAGTAGTT	RT-qPCR
omcB-rtR	CACGCTGTCCAGAAGAATGA	
euo-rtF	TCAAGGAGAGCTTCTGTTTGATAAC	RT-qPCR
euo-rtR	TGCGTGTAGCATAGTAAATCTTCTG	
scc1-rtF	CTTGGAAAACTTCCAGAGAATACCT	RT-qPCR
scc1-rtR	TGGTTAGAGATTTCACCGTATCCTA	
sctU-rtF	CTGCGATTACGTTCATTGTCT CC	RT-qPCR
sctU-rtR	ATGATTCTGGGGAGCTGTTTTAAA	
slc1-rtF	ACACTCTGATCGCCTTTATGTTTAC	RT-qPCR
slc1-rtR	GCATAGAGCCTTCTAACAACTTCTC	
scc1-rtF	CTTGGAAAACTTCCAGAGAATACCT	RT-qPCR
scc1-rtR	TGGTTAGAGATTTCACCGTATCCTA	
incD-rtF	CTCTGTAGCCCTGTTTCTGTTTTAG	RT-qPCR
incD-rtR	CTAGTCACAGCTTCTGTAGTCAGCA	
*scc4*F	TTTAAACTAGAGATGAGCACGTGAATACA	PCR
mcherryR1	TAAATCCTTCTGGAAAAGAGAGCTT	Sequencing

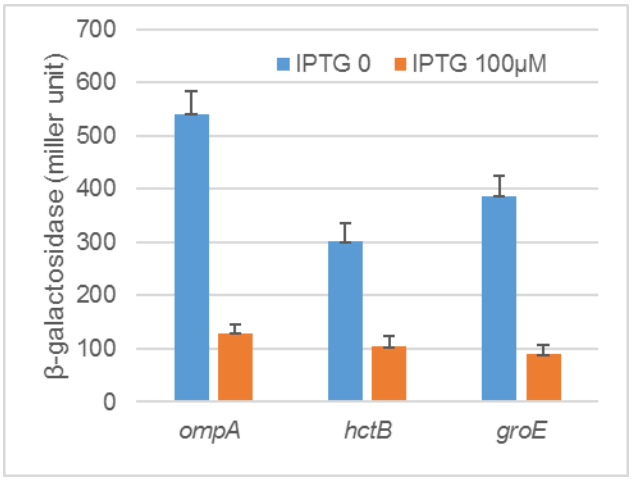
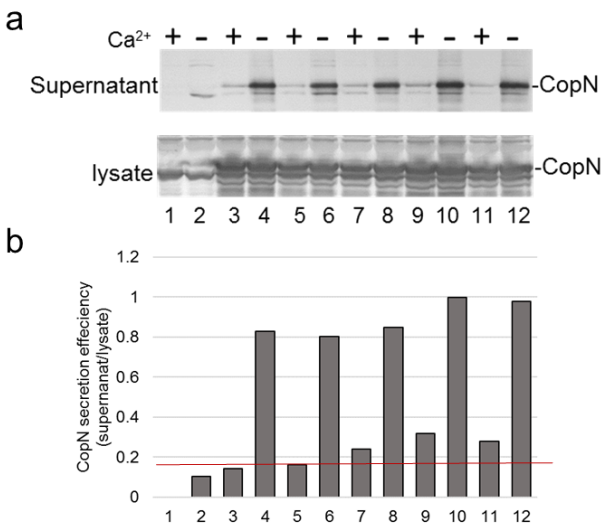


Figure S1. Analysis of promoter activity using β-galactosidase reporter assay. The overnight cultures *E. coli* BL21(DE3) cells carrying pCDFScc4 were diluted in fresh medium at the ratio of 1:100 and cultured in LB broth at 37 °C. When bacteria grown to OD_{600}=0.2, isopropyl-β-D-thiogalactopyranoside (IPTG) was added to induce Scc4 expression. The control was cultured in the absence of IPTG. The cultures were harvested for β-galactosidase reporter assay with the method described previously (3). The data are presented as mean ± SD of β-galactosidase (miller Units) from one representative experiment with three repeats. At least three independent experiments were performed.



1-2. YP1283
3-4. YP1283 + pBAD24-CopN
5-6. YP1283 + pBAD24-CopN + pBAD33-Scc4
7-8. YP1283 + pBAD24-CopN + pBAD33-RBS-Scc4
9-10. YP1283 + pBAD24-CopNScc1
11-12 YP1283 + pBAD24-CopNScc1 + pBAD33-Scc4

Figure S2 CopN secretion assays using YP1283. (**a**) immunoblot analysis of CopN secretion. *Y. pestis* was cultivated in HIB medium in the presence (+) or absence (-) of 2.5 mM Ca^{2+}. After an initial 2-h incubation at 26 °C, IPTG (100 μM) was added. Bacterial cultures were then shifted to 37 °C and continued culturing for 4 h prior to harvesting. Samples representing culture supernatant and bacterial lysates were resolved in 12% polyacrylamide gels and analyzed by immunoblotting with an anti-CopN antibody, followed by visualization of protein by probing with alkaline phosphatase-conjugated secondary antibodies. (**b**) Ratio of secreted CopN levels (in supernatants) to the intracellular levels (lysates). Protein blot in (**a**) was measured using ImageJ. The red line represent the background.

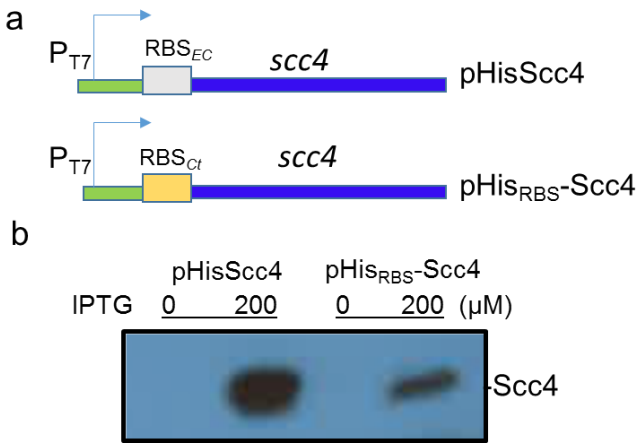


Figure S3 *C. trachomatis scc4* ribosome-binding site (RBS) weakly functions to direct the initiation of Scc4 synthesis in *E. coli.* (**a**) Schematic diagram showing the cloning strategy to construct pET15b-derived expression vectors: pHisScc4 containing an optimal *E. coli* RBS and pHisRBS-Scc4 containing the *C. trachomatis* RBS upstream of *scc4*. (**b**) Immunoblot analysis of Scc4 expression in *E. coli*. BL21(DE3) cells were transformed with pHisScc4 or pHisRBS-Scc4 and cultured in LB. IPTG (200 μM) was added to induce protein expression. The protein samples were resolved in 10% (wt/vol) polyacrylamide gels and analyzed by immunoblotting with an anti-Scc4 antibody, followed by visualization of protein by probing with alkaline phosphatase-conjugated secondary antibodies. The protein samples from bacteria culture without IPTG addition were used as the controls.

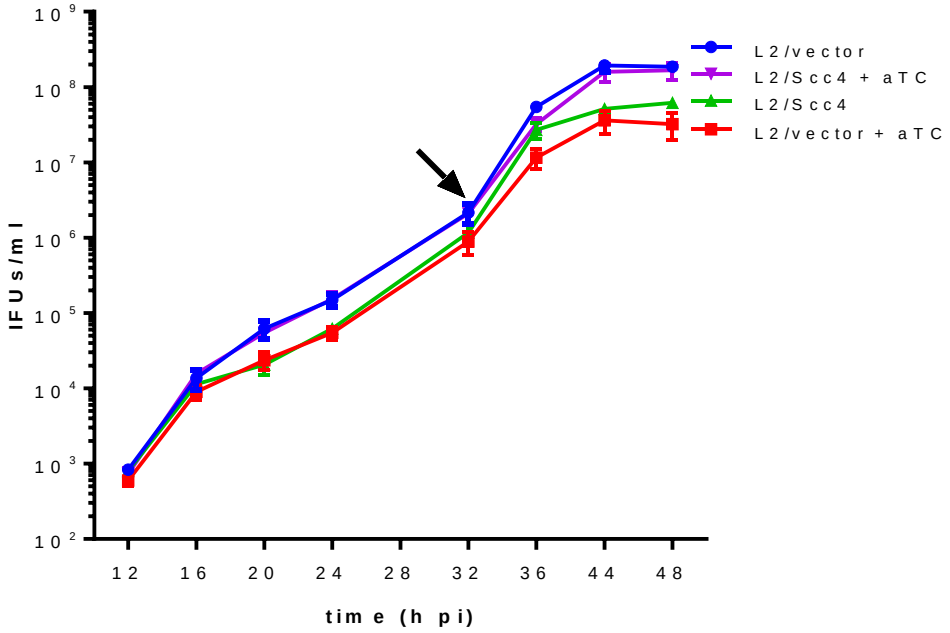


Figure S4 End-point growth curves of *C. trachomatis* measured by inclusion forming unit assay (IFU assay) of infectious EBs. Infected HeLa cells were sampled for analysis at different times as indicated. The aliquot of dilutions were inoculated onto fresh monolayer HeLa cells and cultured for 40 h. Inclusions were then stained with a mouse monoclonal primary antibody against LGV L2 MOMP, followed by staining with goat anti-mouse IgG Alexa Fluor 567-conjugate secondary antibody. The quantity of inclusion forming units were counted and converted to IFUs/ml. All values are presented as means ± SD of triplicate samples. An arrow shows the close similarities of IFUs among each strain at 32 h pi.

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