Supporting Information

Identification of the DotL Coupling Protein Subcomplex of the *Legionella* Dot/Icm Type IV Secretion System

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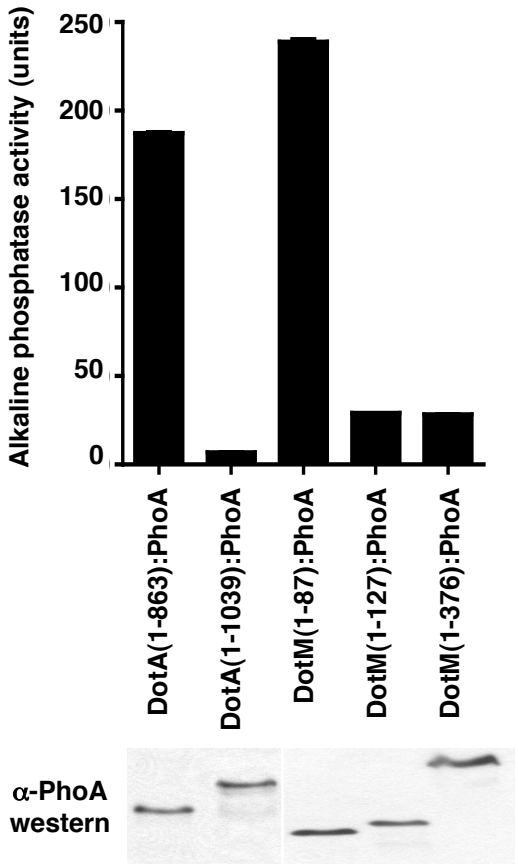

Figure S1. Membrane Topology of DotL and DotM.

Topology of DotM was experimentally determined using fusions to the reporter alkaline phosphatase (PhoA). PhoA is active when present in the periplasm, but is inactive when it localizes to the cytoplasm. Three DotM-PhoA fusions were constructed based on the predicted transmembrane domains of DotM using the Kyte-Doolittle hydrophilicity prediction program. A PhoA fusion after aa 87 of DotM was positive whereas fusions after aa 127 and aa 376 (the end of the protein) were negative. Equivalent production of the PhoA fusion proteins were confirmed by western blotting using an alkaline phosphatase antibody (Chemicon International). Fusions to the inner membrane protein DotA were used as controls. Controls included fusion of the first 863 aa of DotA as a PhoA-positive and fusion of the first 1039 aa as a PhoA-negative as previously described (Roy & Isberg, 1997). Results shown are representative of three independent experiments.

	X:T18	T25:X
1	Zip	Zip
2	Vector	Vector
3	DotM(all)	Vector
4	DotM(1-220)	Vector
5	DotM(1-127)	Vector
6	Vector	DotL(3-73)
7	DotM(all)	DotL(3-73)
8	DotM(1-220)	DotL(3-73)
9	DotM(1-127)	DotL(3-73)

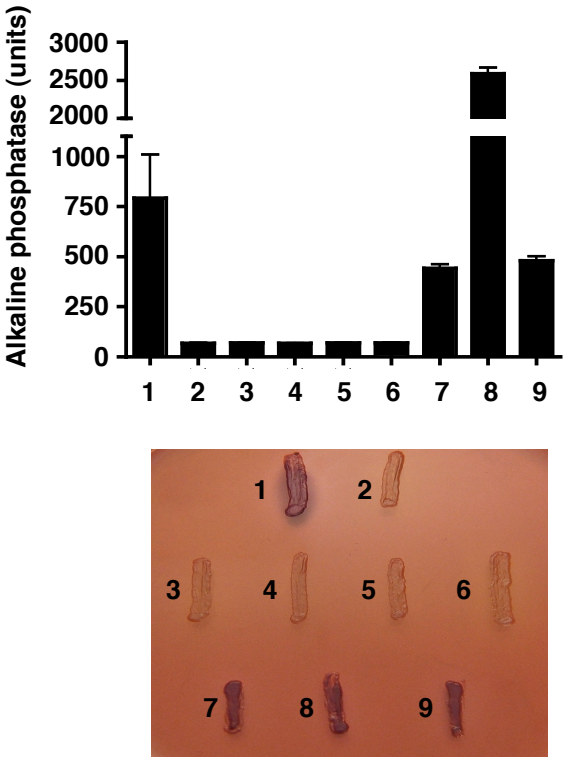

Figure S2. Two-Hybrid Analysis of the DotL-DotM Interaction.

The Ladant bacterial two-hybrid system is based on reconstitution of adenylate cyclase activity when the T18 and T25 domains of CyaA are brought into proximity via fusions to two proteins that interact. A positive signal produces cAMP, which can be quantified by increased β-galactosidase activity (upper right) and the fermentation of maltose on a MacConkey agar plate (red color in photo shown on lower right). The strains assayed are shown in the table on the left. The positive control (strain 1) consists of two leucine zipper domains fused to the T18 and T25 domains of CyaA. The negative control (strain 2) consists of the empty T18 and T25 fusion vectors. Experimental strains consisted of fusions to the first 73 amino acids of DotL and fusions of the first 127, 220, and 326 amino acids of DotM. The positive signal detected with DotL and DotM fusions was specific as each fusion assayed with the corresponding empty vector was negative. Results shown are representative of at least three independent experiments.

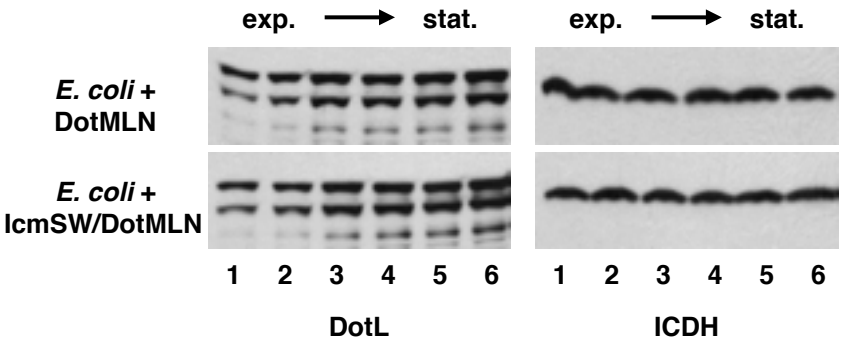

Figure S3. Stability of DotL in the Presence and Absence of IcmS and IcmW in *E. coli*. *E. coli* KY1429 strains were grown in broth with 0.1 mM IPTG and cells were collected at various stages of growth. Whole-cell extracts were analyzed by Western blotting using antibodies specific to the proteins listed beneath each panel. In each panel, lanes contain extracts made from cultures at the following OD_{600}: lane 1, OD_{600} ~0.6, lane 2, ~1.0, lane 3, ~1.3, lane 4, ~1.5, lane 5, ~1.6, and lane 6, ~1.7 ("exp" stands for exponential phase and "stat" represents stationary phase). Strains used were: KY1429 plus pJB4632 (*dotM dotL dotN*) and KY1429 plus pJB4636 (*icmS icmW dotM dotL dotN*). Western blots for ICDH served as a loading control. Results shown are representative of three independent experiments.

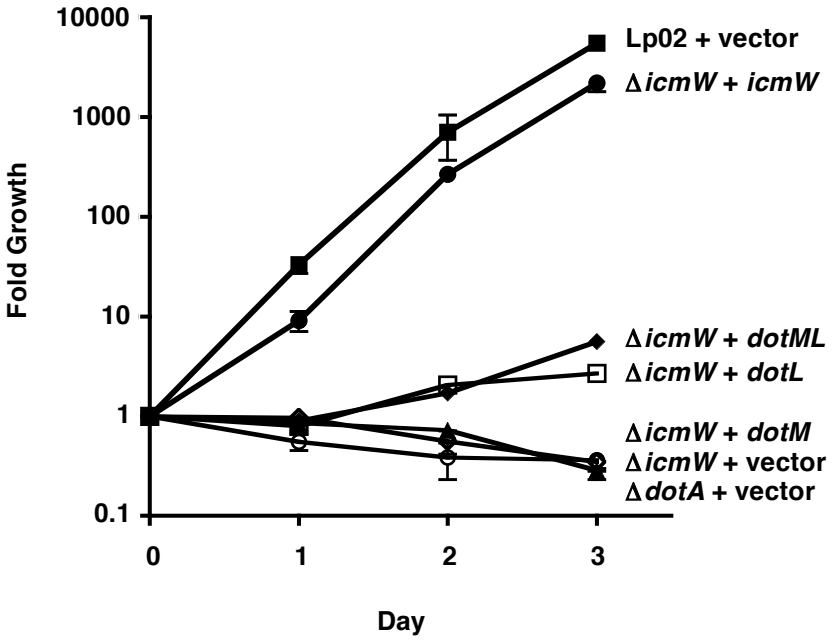

Figure S4. Partial Complementation of the Δ*icmW* Mutation by Overexpression of DotL. Strains used were: wild-type plus vector (JV1139, filled squares), Δ*icmW* plus *icmW* (JV3649, filled circles), Δ*icmW* plus *dotM* + *dotL* (JV5571, filled diamonds), Δ*icmW* plus *dotL* (JV5570, open squares), Δ*icmW* plus *dotM* (JV5880, open diamonds), Δ*icmW* plus vector (JV3658, open circles), and Δ*dotA* plus vector (JV3029, filled triangles). Fold growth was calculated by dividing the number of colony forming units (CFU) recovered each day by the number of CFU recovered immediately after infection (day 0). Each time point represents the mean and standard deviation of colony forming units (CFU) recovered from triplicate wells. Growth curves are representative of three independent experiments.

Table S1. Bacterial strains, plasmids, and primers employed in this study

Strain, plasmid or primer	Relevant properties	Reference or source
L. pneumophila		
Lp02	Philadelphia-1 *thyA, hsdR, rpsL*	(Berger & Isberg, 1993)
JV1139	Lp02 + pJB908	(Bardill *et al*., 2005)
JV1962	Lp02 Δ*icmS*	(Vincent & Vogel, 2006)
JV2064	Lp02 Δ*dotA*	(Vincent & Vogel, 2006)
JV2422	Lp02 Δ*dotA* Δ*dotL*::Cm^R	(Buscher *et al*., 2005)
JV3029	JV2064 + pJB908	This study
JV3598	Lp02 Δ*icmW*	(Vincent & Vogel, 2006)
JV3649	JV3598 + pJB2875	This study
JV3658	JV3598 + pJB908	This study
JV3719	Lp02 Δ*dotA* Δ*dotN*	(Vincent *et al*., 2006)
JV4044	Lp02 Δ*dot/icm* complete	(Vincent et al., 2006)
JV4191	JV4044 + pJB908	This study
JV4192	JV4044 + pJB3250	This study
JV4193	JV4044 + pJB3248	This study
JV4194	JV4044 + pJB1014	This study
JV5156	JV1962 + pJB908	This study
JV5157	JV1962 + pJB4271	This study
JV5361	Lp02 Δ*dotA* Δ*dotM*	This study
JV5565	JV1962 + pJB1014	This study
JV5566	JV1962 + pJB3250	This study
JV5570	JV3598 + pJB1014	This study
JV5571	JV3598 + pJB3250	This study
JV5775	Lp02 Δ*ldsB*	This study
JV5809	JV1962 + pJB3248	This study
JV5880	JV3598 + pJB3248	This study
JV5972	Lp02 Δ*dotN* Δ*ldsB*	This study
JV5976	Lp02 Δ*dotL* Δ*ldsB*	This study
JV6019	Lp02 Δ*dotM* Δ*ldsB*	This study
JV6315	Lp02 Δ*clpA*::CmR	This study
JV6339	Lp02 Δ*icmS* Δ*clpA*::Cm^R	This study
JV6294	JV6339 + pJB4897	This study
JV6296	JV6339 + pJB908	This study
JV6503	JV6315 + pJB908	This study
E. coli		
BTH101	*cya*-99, *araD139*, *galE15*, *galK16* *rpsL1* (StrR), *hsdR2*, *mcrA1*, *mcrB1*	(Karimova *et al*., 1998)

KY1429	*araD139 argF-lac169 flhD5301 fruA25 relA1 rpsL150 (strR) zhh-50*::Tn*10 rpoH606 (ts) deoC1*	(Tobe *et al*., 1984)
JB3188	KY1429 + pJB908	This study
JB3189	KY1429 + pJB1014	This study
JB3190	KY1429 + pJB3248	This study
JB3191	KY1429 + pJB3250	This study
JB4728	KY1429 + pJB4610	This study
JB4729	KY1429 + pJB4632	This study
JB4730	KY1429 + pJB4636	This study

Plasmids

pBluescript II KS(+)	ColE1 cloning vector	Stratagene
pJB908	RSF1010 cloning vector	(Sexton *et al*., 2004)
pJB1014	pJB908 + *dotL*	(Buscher et al., 2005)
pJB1806	pJB908 CmR, without *tdΔI*	(Bardill *et al*., 2005)
pJB2875	pJB908 + *icmW*	This study
pJB3248	pJB908 + *dotM*	This study
pJB3250	pJB908 + *dotM dotL*	This study
pJB3270	pJB1806 + PhoA fusion vector	This study
pJB3322	Ladant T25:X fusion (pUC MCS)	This study
pJB3656	Ladant X:T18 fusion (pUC MCS)	This study
pJB3671	DotM(1-127):T18	This study
pJB3672	DotM(1-376):T18	This study
pJB3765	pJB3270 + DotM(1-127):PhoA	This study
pJB3769	pJB3270 + DotM(1-376):PhoA	This study
pJB3771	pJB3270 + DotM(1-87):PhoA	This study
pJB4150	pJB3270 + DotA(1-1039):PhoA	This study
pJB4271	pJB908 + *icmS*	This study
pJB4299	Bluescript II KS(+) with pUC MCS	This study
pJB4331	pJB3270 + DotA(1-863):PhoA	This study
pJB4570	Δ*ldsB* suicide plasmid	This study
pJB4610	pJB908 + *icmS icmW dotM dotL*	This study
pJB4632	pJB908 + *dotM dotL dotN*	This study
pJB4636	pJB908 + *icmS icmWdotM dotL dotN*	This study
pJB4680	Δ*clpA* suicide vector	This study
pJB4712	T25:DotL(3-73)	This study
pJB4722	DotM(1-220):T18	This study
pJB4833	Δ*clpA*::Cm^R marked suicide plasmid	This study
pJB4897	*clpA* complementing clone	This study
pKRP10	Cm^R encoding gene	(Reece & Phillips, 1995)
pSR47S	R6K suicide vector (Kan^R, *sacB*)	(Merriam *et al*., 1997)
pT18	bacterial 2-hybrid vector	(Karimova *et al*., 1998)
pT18-zip	bacterial 2-hybrid positive control	(Karimova et al., 1998)
pT25	bacterial 2-hybrid vector	(Karimova et al., 1998)
pT25-zip	bacterial 2-hybrid positive control	(Karimova et al., 1998)

Primers

JVP274	GCATTCTTGATGAGTACGG
JVP618	GCTCCAAAACTTGGGATACC
JVP981	CCCGTCGACCCTGTTCTGGAAAACCGGGC
JVP1058	CCCGGATCCAGGAGAAATTACTATGCCTGATTTAAGCCATGAAGC
JVP1059	CCCGTCGACGGTAATACTTTCATTATTCATCCC
JVP1154	CCCGGTACCCGCAAAAATAGCTCTGAGCAG
JVP1155	CCCGCATGCCTATTTCATGGCTCTAATTCCTCCATTTGACG
JVP1177	CCCCTCGAGCTATTTCAGCCCCAGAGCGGCTTTCATGG
JVP1225	CTTGGATCCCCCGGGCTCGAGCTGCAGGCATGCGGCCGCGGTAC
JVP1226	ACGTGAACCTAGGGGGCCCGAGCTCGACGTCCGTACGCCGGCGC
JVP1427	CCCGGATCCCCGCAAACTTTTCATATCATAGG
JVP1431	CCCGGATCCTGGCTCTAATTCCTCCATTTGACG
JVP1432	CCCGGTACCAGGAGAAATTAGAGATGGCACAACAACAGCAGC
JVP1462	CCCGGATCCACGCATTACAGTCACCATTTGATCC
JVP1429	CCCGGATCCTGCGTAAGCCTGAGGAGAGCAACG
JVP1547	CGCGAGCTCAGGAGAAATTAACTATGAATAAATTAGCTATTACGGTCCTC
JVP1550	CGCTCTAGAACCACTATCAGAACCGCCCAG
JVP1570	GCGGTCGACGCCGCCAGCGAGGCCACGGG
JVP1571	GCAGCGGCCGCCTAGCGTTCCACTGCGCCCAGCG
JVP1575	CGCGGATCCCGGGGTATTGATTCTCGTCATG
JVP1579	GATAGGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACGCGGCCGCAGCT
JVP1580	GCGGCCGCGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCCTATCGTAC
JVP1585	CGCTCTAGACTTCGCATCATTCGAACTGGTGC
JVP1693	GCGGTCGACCTTCCAGCATTGAGTACTGG
JVP1694	CGCGGATCCGTATCTGAAAGGTATAACTTGC
JVP1695	CGCGGATCCGCCCGTTATTTGGATTTATAGGC
JVP1696	CGCGCGGCCGCCCAACGTTCCCAGAAAAGG
JVP1704	CCCCTGCAGTTACTTTCGGGTATAGCTATAAAGAAAAAATATGCC
JVP1737	CGCGGTACCGAGGAGAAATTAACTATGGAGCGAGATATTAGCAAG
JVP1738	CGCGGTACCTTATTCATCCCCTTCGAGTGCTCG
JVP1741	CGCGCATGCTTATGTTAATTCCTCTGCAGCC
JVP1791	CCCGTCGACGAGTGAATCATATCTAATCACTATGC
JVP1792	CCCGGATCCTGCTTCCTTGAAAGCTAGATTCAAGG
JVP1793	CCCGGATCCCTGCATTTTGACAGTCATGATCATAGAG
JVP1794	CCCGCGGCCGCGTACCTTGAATCTTGTTTAGCAGG
JVP1877	CCCGAGCTCGGGTTGGTAAGGAGCAATGAC
JVP1878	CCCGGTACCGGGTTTCCGTTTAACAAACACC

*Restriction enzyme sites are underlined

Table S2. Proteases and regulators checked for DotL degradation in the absence of IcmS

Lpg#	Protease class	Predicted localization	*E. coli* homolog	% Identity
0019	Zinc metalloprotease	Periplasm	n.h.*	--
0499	Serine	Periplasm	Prc/Tsp	28
0903	Serine	Periplasm	DegP (Do-like)	35
0825	Zinc metalloprotease	Periplasm	YebA	44
1655	Zinc metalloprotease	Periplasm	n.h.	--
2019	Serine	Periplasm	n.h.	--
2671	Zinc metalloprotease	Periplasm	PqqL	25
2672	Zinc metalloprotease	Periplasm	PqqL	24
0180	Zinc metallo.	Inner memb.	HtpX	30
0505	Zinc metallo.	Inner memb.	RseP	43
0855	Serine	Inner memb.	Signal peptide peptidase	46
1176	Zinc metallo.	Inner memb	YfgC	32
1525	Unknown	Inner memb.	CAAX N-terminal protease	--
1749	Serine	Inner memb.	Signal peptide peptidase	27
2007	Aspartyl	Inner memb.	n.h.	--
2333	Zinc metallo.	Inner memb.	RseP	30
2586	Cysteine	Inner memb.	n.h.	--
2622	Cysteine	Inner memb.	n.h.	--
2978	Zinc metallo.	Inner memb.	n.h.	--
0392	Zinc metallo.	Cytoplasm	n.h.	--
0640	Threonine	Cytoplasm	HslV	82
1859	Serine	Cytoplasm	La (Lon)	68
1977	Cysteine	Cytoplasm	ThiJ/PfpI	29
2977	Zinc metallo.	Cytoplasm	n.h.	--
0818	Regulator	Cytoplasm	ClpA	66
1438	Regulator	Cytoplasm	CpxR	48
1577	Regulator	Cytoplasm	RpoE	36

*n.h. stands for no significant homology

Supplementary Materials and Methods

Construction of plasmids. The following clones were constructed as described below. Primers (named JVP) are listed in Table S1. PCR inserts were sequenced for all plasmids to confirm that no errors were introduced during amplification.

pJB2875 (*icmW* complementing clone) was constructed by amplifying *icmW* using JVP1058/JVP1059, digesting the PCR product with BamHI/SalI and cloning it into BamHI/SalI-digested pJB908.
pJB3248 (*dotM* complementing clone) was constructed by amplifying *dotM* using JVP1154/JVP1155, digesting the PCR product with KpnI/SphI and cloning it into KpnI/SphI-digested pJB908.
pJB3250 (*dotM*/*dotL* complementing clone) was constructed by amplifying *dotM* and the amino-terminus of *dotL* using JVP1154/JVP618, digesting the PCR product with KpnI/XhoI and cloning it into KpnI/XhoI-digested pJB1014.
pJB3270 (X:PhoA fusion vector) was constructed by amplifying *phoA* using JVP981/JVP1177, digesting the PCR product with SalI/XhoI and cloning it into SalI-digested pJB1806.
pJB3322 (Ladant T25:X fusion vector) was constructed by annealing primers JVP1225/JVP1226 together and ligating them into PstI/KpnI-digested Ladant vector pKT25.
pJB3656 (Ladant X:T18 vector with pUC MCS) was constructed by amplifying a portion of *cyaA* from pT18 using JVP1570/JVP1571, digesting the PCR product with SalI/NotI and cloning it into SalI/NotI-digested pJB4299.
pJB3671 (DotM(1-127):T18 fusion) was constructed by subcloning a KpnI/BamHI fragment containing a portion of *dotM* from pJB3765 into KpnI/BamHI-digested pJB3656 (X:T18 Ladant fusion vector).
pJB3672 (DotM(all):T18 fusion) was constructed by subcloning a KpnI/BamHI fragment containing *dotM* from pJB3656 into KpnI/BamHI-digested pJB3656 (X:T18 Ladant fusion vector).
pJB3765 (DotM(1-127):PhoA) was constructed by amplifying a portion of *dotM* using JVP1427/JVP1432, digesting the PCR product with KpnI/BamHI and cloning it into KpnI/BamHI-digested pJB3270 (X:PhoA fusion vector).

pJB3769 (DotM(all):PhoA) was constructed by amplifying a portion of *dotM* using JVP1431/JVP1432, digesting the PCR product with KpnI/BamHI and cloning it into KpnI/BamHI-digested pJB3270 (X:PhoA fusion vector).

pJB3771 (DotM(1-87):PhoA) was constructed by amplifying a portion of *dotM* using JVP1432/JVP1462, digesting the PCR product with KpnI/BamHI and cloning it into KpnI/BamHI-digested pJB3270 (X:PhoA fusion vector).

pJB4150 (DotA(1-1039):PhoA) was constructed by amplifying a portion of *dotM* using JVP1547/JVP1550, digesting the PCR product with SacI/XbaI and cloning it into SacI/XbaI-digested pJB3270 (X:PhoA fusion vector).

pJB4271 (*icmS* complementing clone) was constructed by subcloning a EcoRI/BamHI fragment containing *icmS* from pJB1745 into EcoRI/BamHI-digested pJB1550 (modified pJB908 vector lacking the 5' EcoRI site).

pJB4299 (Bluescript II KS(+) with pUC MCS was constructed by annealing primers JVP1579/JVP1580 together and ligating them into KpnI/SacI-digested Bluescript II KS(+).

pJB4331 (DotA(1-863):PhoA) was constructed by amplifying a portion of *dotM* using JVP1547/JVP1585, digesting the PCR product with SacI/XbaI and cloning it into SacI/XbaI-digested pJB3270 (X:PhoA fusion vector).

pJB4570 (Δ*ldsB* suicide vector) was constructed by amplifying 500 bp upstream and 500 bp downstream of the *ldsB* ORF using primers JVP1693/JVP1694 and JVP1695/JVP1696. The PCR products were digested with SalI/BamHI and BamHI/NotI, respectively, and cloned into SalI/NotI-digested pSR47S.

pJB4610 (*icmS*/*icmW*/*dotM*/*dotL* complementing clone) was constructed by amplifying *icmS*/*icmW* operon using JVP1737/JVP1738, digesting the product with KpnI and cloning it into KpnI-digested pJB3250 (*dotM*/*dotL* complementing clone).

pJB4632 (*dotM*/*dotL*/*dotN* complementing clone) was constructed by PCR using primers JVP274/JVP1741, followed by subcloning the BglII/SphI-digested fragment into BglII/SphI-digested pJB3692, thus generating pJB4624. This was followed by subcloning the *dotN* ORF from pJB3692 on a SphI fragment into SphI-digested pJB4624.

pJB4636 (*icmS*/*icmW*/*dotM*/*dotL*/*dotN* complementing clone) was constructed by subcloning a KpnI/KpnI fragment from pJB4610 containing *icmS*/*icmW* into KpnI-digested pJB4632 (*dotM*/*dotL*/*dotN* complementing clone).

pJB4680 (Δ*clpA* suicide vector) was constructed by amplifying 500 bp upstream and 500 bp downstream of the *clpA* ORF using primers JVP1791/JVP1792 and JVP1793/JVP1794. The PCR products were digested with SalI/BamHI and BamHI/NotI, respectively, and cloned into SalI/NotI-digested pSR47S.

pJB4712 (T25:DotL(3-73stop) was constructed by amplifying a portion of *dotL* using JVP1575/JVP1704, digesting the PCR product with BamHI/PstI and cloning it into BamHI/PstI-digested pJB3322 (T25:X Ladant vector).

pJB4722 (DotM(1-220):T18 fusion) was constructed by subcloning a KpnI/BamHI fragment containing a portion of *dotM* from pJB3767 into KpnI/BamHI-digested pJB3656 (X:T18 Ladant fusion vector).

pJB4833 (Δ*clpA*::Cm^R marked suicide vector) was constructed by subcloning a BamHI/BamHI fragment containing a chloramphenicol resistance cassette from pKRP10 into BamHI-digested pJB4680 (Δ*clpA* suicide vector).

pJB4897 (*clpA* complementing clone) was constructed by amplifying *clpA* using JVP1877/JVP1878, digesting the PCR product with SacI/KpnI and cloning it into SacI/KpnI-digested pJB908.

Generation of *L. pneumophila* mutants:

JV5775 (Lp02 Δ*ldsB*) was constructed by transforming Lp02 with pJB4570 (Lp02 Δ*ldsB*), selecting for integrants on CYE plates supplemented with thymidine (100 μg/ml) and kanamycin (30 μg/ml), following by resolution of the merodiploid by growth on CYE plates supplemented with thymidine and sucrose (5%). Deletions were identified by PCR. JV5972 (Δ*ldsB* Δ*dotN*), JV5976 (Δ*ldsB* Δ*dotL*), and JV6019 (Δ*ldsB* Δ*dotL*) were constructed in a similar manner by integration and resolution of pJB3046 (Δ*dotN* suicide plasmid), pJB1001 (Δ*dotL* suicide plasmid), pJB4429 (Δ*dotM* suicide plasmid), respectively, into JV5775 (Lp02 Δ*ldsB*).

The Δ*clpA*::Cm^R insertion mutants ((JV6315 (Lp02 Δ*clpA*::Cm^R) and JV6339 (Lp02 Δ*icmS* Δ*clpA*::Cm^R)) were constructed by natural transformation of Lp02 or JV1962 (Δ*icmS*), respectively, using pJB4833 (Δ*clpA*::Cm^R marked suicide plasmid). Natural transformation was done as previously described (Sexton & Vogel, 2004). Transformants were selected on CYET + Cm plates.

Alkaline phosphatase assays. Plasmids pJB3771 (DotM(1-87):PhoA), pJB3765 (DotM(1-127):PhoA), and pJB3769 (DotM(1-376):PhoA were generated by amplifying *dotM* with primers JVP1462/JVP1432, JVP1427/JVP1432, and JVP1431/JVP1432, respectively, and cloning the PRC product into pJB3270. *L. pneumophila* strains containing PhoA fusion proteins were grown to stationary phase in AYE medium and induced in exponential phase for two hours at 37° C with IPTG (100 μM) to induce expression of the fusion proteins. Alkaline phosphatase activity was measured as previously described (Roy & Isberg, 1997, Vincent et al., 2006).

Ladant bacterial two-hybrid screen. Plasmid pJB4712 (T25:DotL(3-73)) was generated by amplifying *dotL* with primers JVP1575/JVP1704 and cloning the PCR product into pT25 (Karimova et al., 1998). Plasmids pJB3671 (DotM(1-127):T18), pJB4722 (DotM(1-220):T18), and pJB3672 (DotM(1-376):T18) were generated by amplifying *dotM* with primers JVP1427/JVP1432, JVP1429/JVP1432, and JVP1431/JVP1432, respectively, and cloning the PRC product into pT18 (Karimova et al., 1998). Interactions were assayed by plating transformants of the *E. coli cyaA* mutant BTH101 on MacConkey maltose (1%) plates at room temperature (Karimova et al., 1998). β-galactosidase production was assayed as previously described (Vincent & Vogel, 2006).

Intracellular targeting assays. Bacteria were grown in AYE to early stationary phase, washed with sterile water, diluted in RPMI and added to mouse bone marrow-derived macrophages. Infections were allowed to proceed for 1 hour at 37° C in 5% CO_2 prior to fixation with paraformaldehyde-lysine-periodate (PLP) containing 5% sucrose. Extracellular bacteria were stained with rabbit anti-*Legionella* IB1 (diluted 1:10,000 in PBS containing 10% goat serum) (Swanson & Isberg, 1995), then stained with goat anti-rabbit secondary antibody

conjugated to Cascade Blue (Molecular Probes) (diluted 1:10,000). The macrophages were then permeabilized with methanol and intracellular bacteria were stained with IB1 and goat anti-rabbit secondary antibody conjugated to Alexa Fluor 594 (Molecular Probes) (diluted 1:10,000). LAMP-1 was stained using the rat monoclonal antibody ID4B (diluted 1:2.5) and goat anti-rat secondary antibody conjugated to Alexa Fluor 488 (Molecular Probes) (diluted 1:100). The ID4B monoclonal antibody developed by J. Thomas August was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by the University of Iowa, Department of Biological Sciences, Iowa City, IA 52242. Co-localization of intracellular bacteria with LAMP-1 was assessed by immunofluorescence microscopy. For each strain four sets of 100 intracellular bacteria were scored; averages and standard deviations are shown.

Intracellular growth assays. *L. pneumophila* strains were grown for 48 hours on CYE plates, resuspended in sterile water to a concentration of 1 x 10^9 cells/ml, and diluted 1:4000 in tissue culture medium. Bacteria were then added to monolayers of U937 cells and incubated for 1 hour at 37° C. Monolayers were then washed, fresh culture medium was added, and the cells were incubated at 37° C in 5% CO_2. To determine the number of colony forming units (CFU) of bacteria present, monolayers were lysed by incubation in sterile water and dilutions were plated on CYE plates. Growth assays were performed in triplicate. Fold growth was calculated by dividing the number of CFU recovered each day after infection by the number of CFU recovered immediately after infection (day 0).

Supporting Information References

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