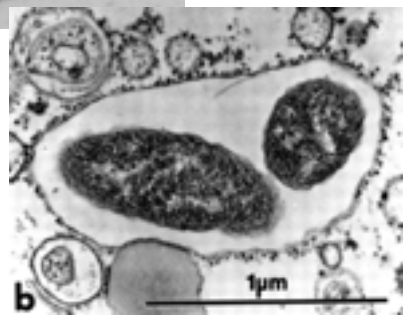
The Dot/Icm Type IV secretion system and effector proteins: essential players in *Legionella* infection.

Hiroki Nagai
RIMD, Osaka University, JAPAN



Legionella pneumophila

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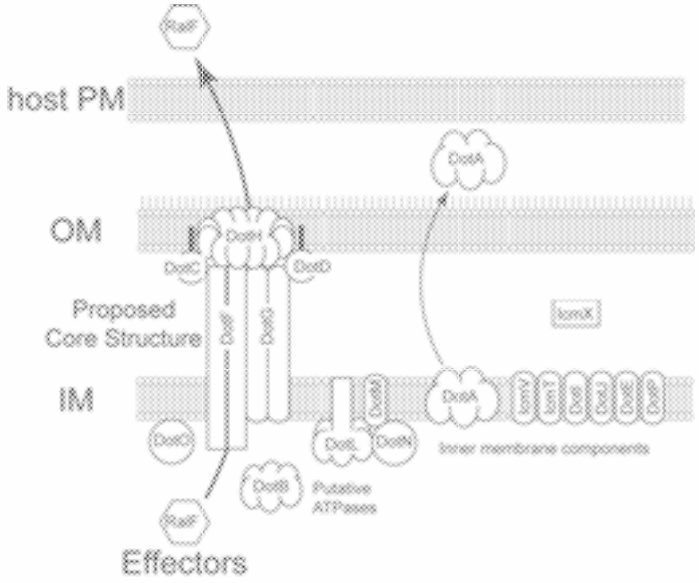

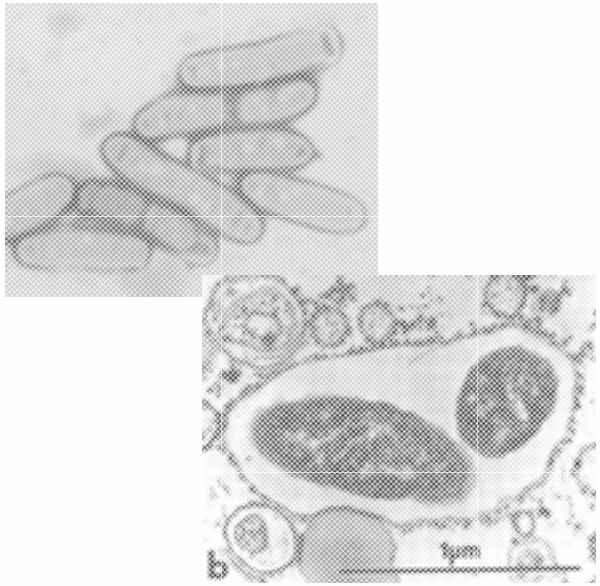

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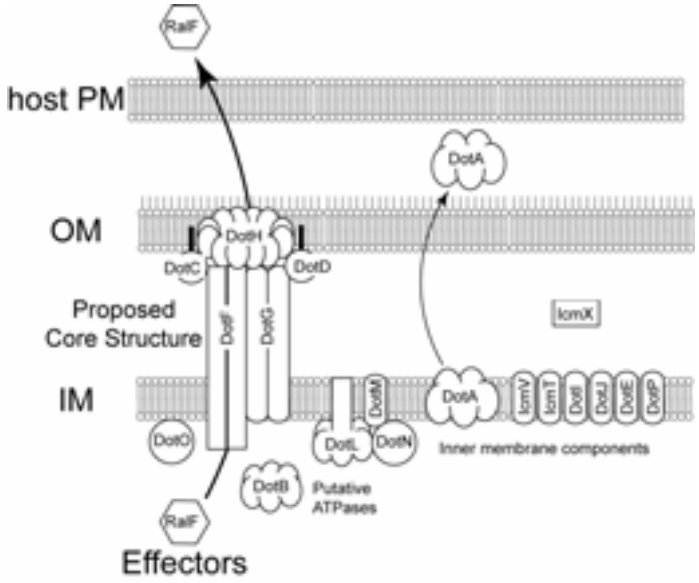
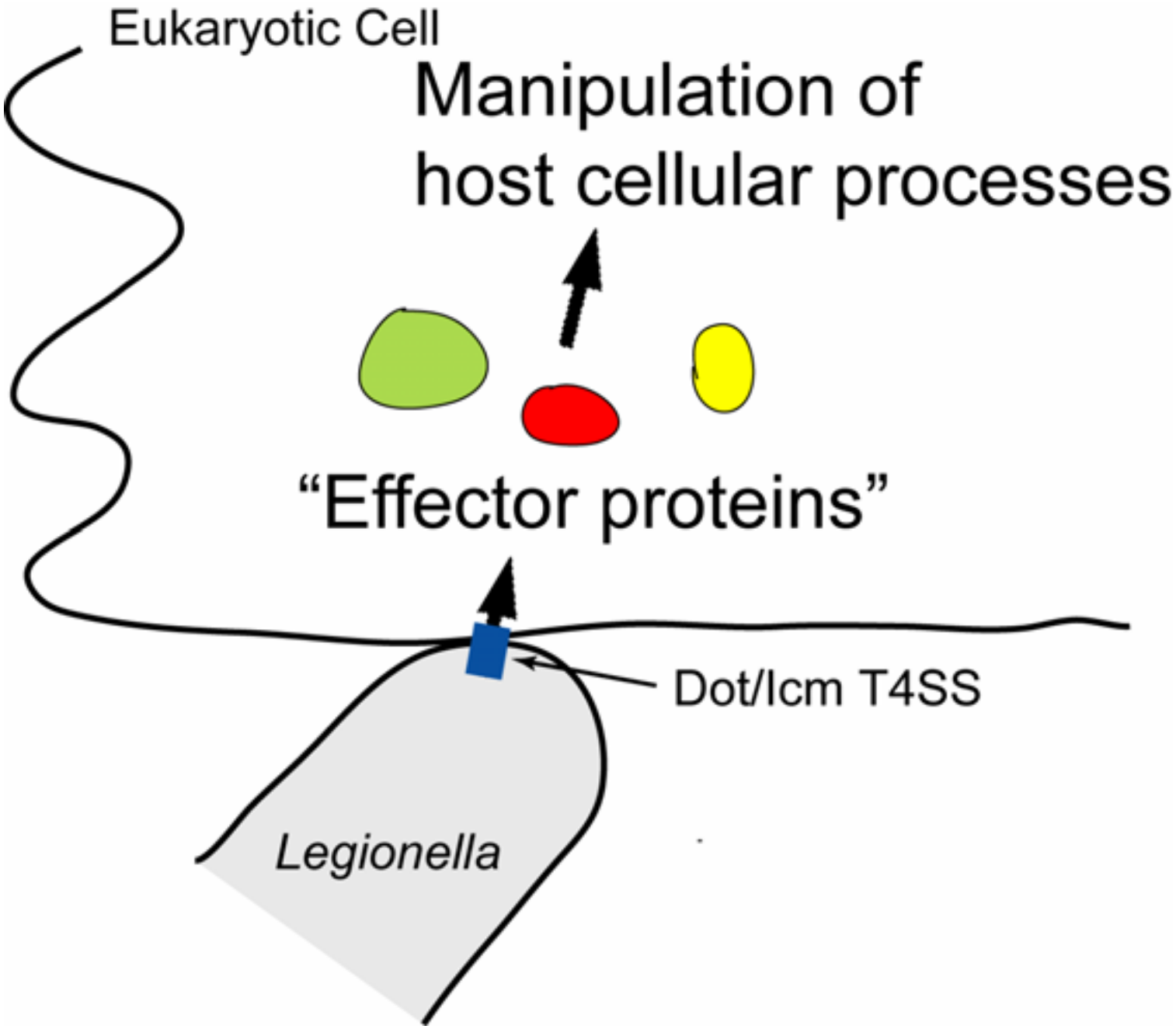
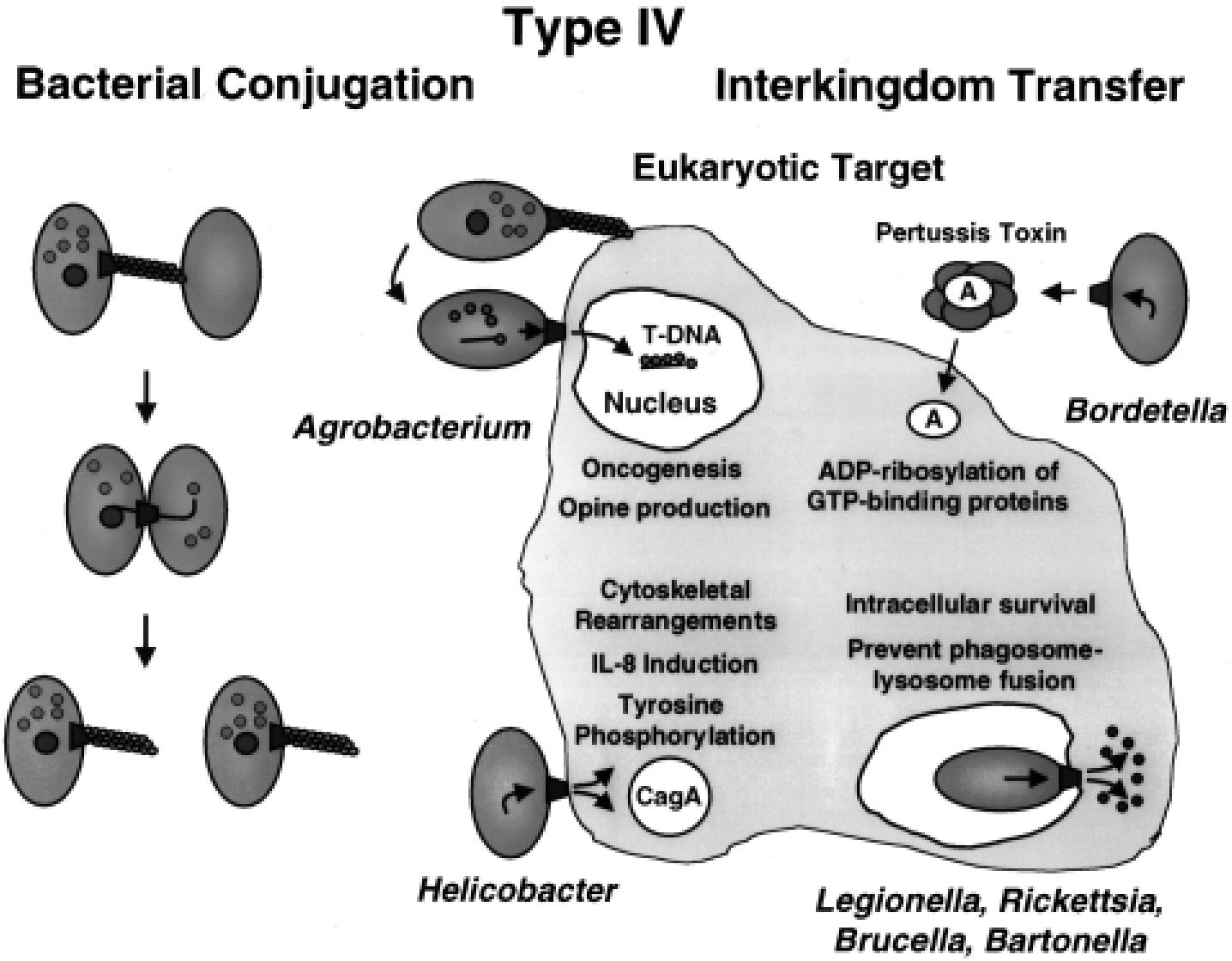
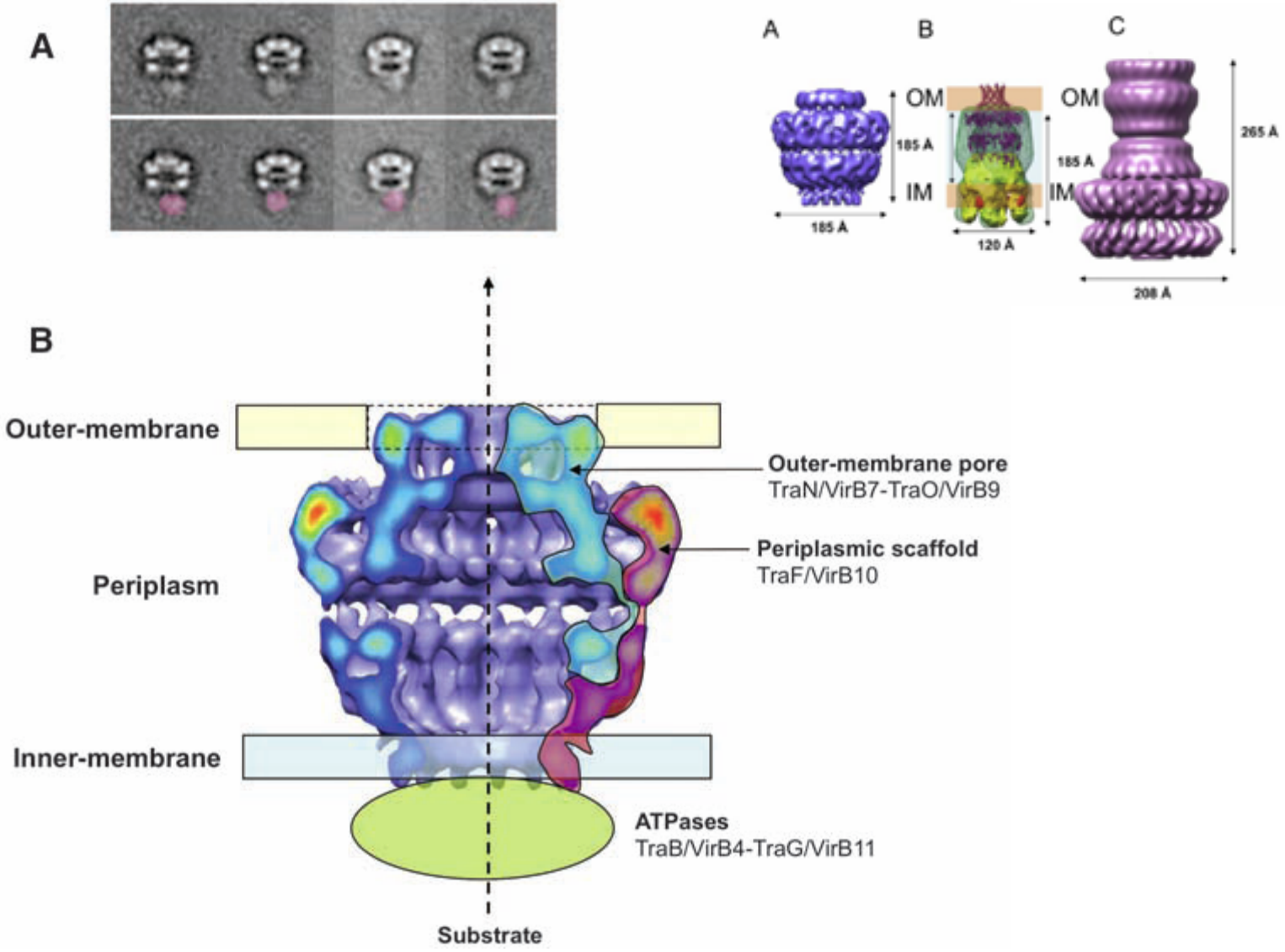



Eukaryotic Cell
Manipulation of
host cellular processes
"Effector proteins"
Dot/Icm T4SS
Legionella



Christie, P. (2001) Mol. Microbiol. **40** (2), 294-305.

The core complex of the pKM101 T4SS

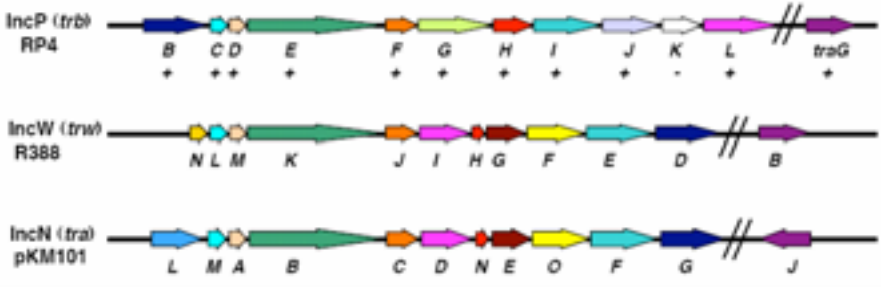


Fronzes et al. *Science* 2009

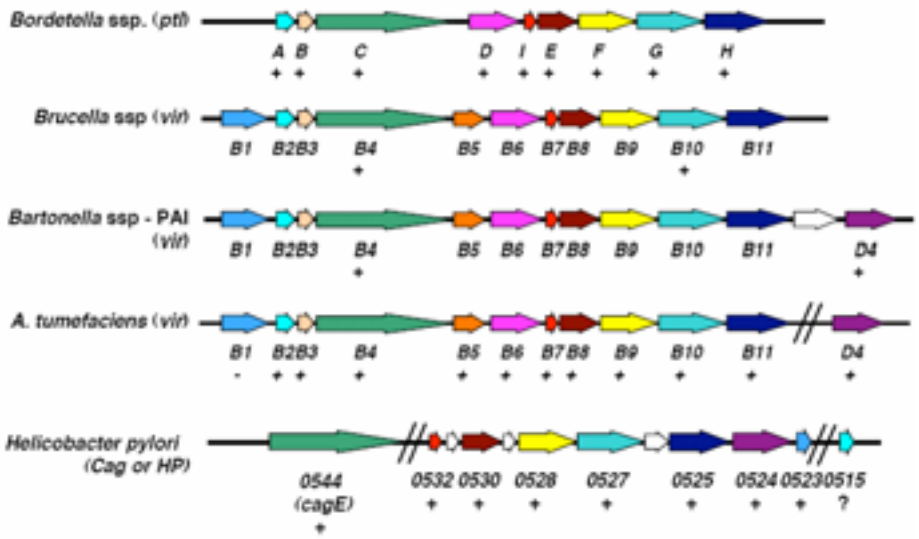
Type IVA

pKM101 plasmid
Agrobacterium tumefaciens
Helicobacter pylori

Conjugation systems

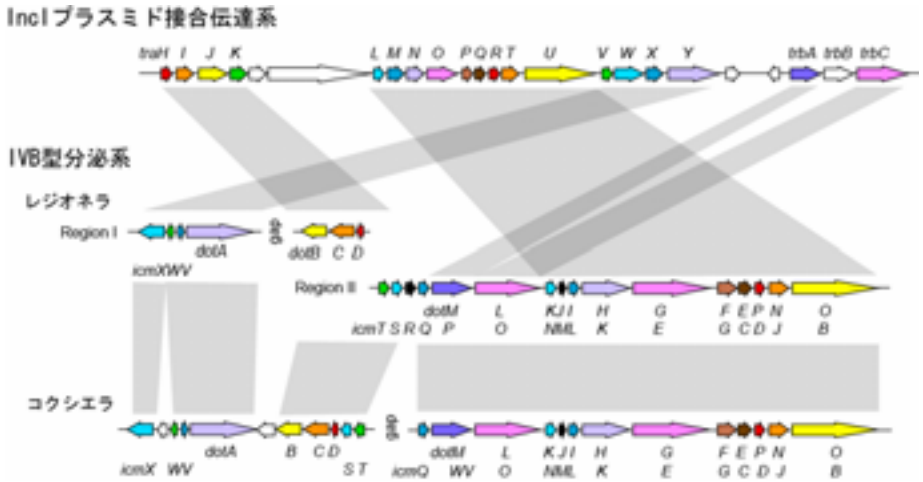


Type IVA secretion systems



Type IVB

Col1b plasmid
Legionella pneumophila
Coxiella burnetii



The *Legionella* Dot/Icm type IV secretion system

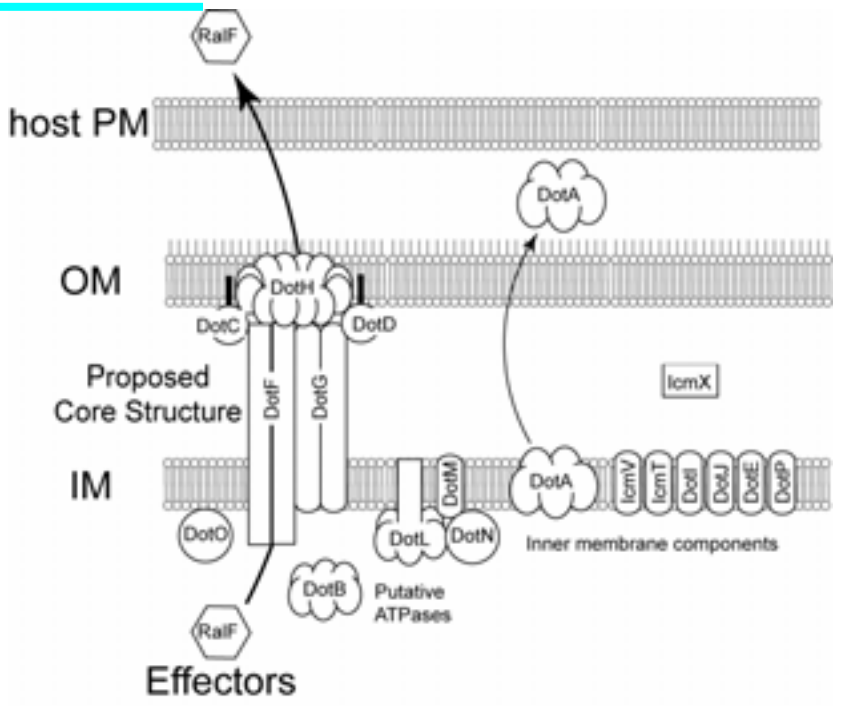
・Putative core components
DotH　forms channel in OM?
DotC　required for DotH sorting
DotD　required for DotH sorting
DotG　forms channel in IM?
DotF　required for efficient core assembly?

・Inner membrane components
DotO/DotP/DotI/DotJ/DotE/IcmT　?
DotL/DotM/DotN　coupling factor and associates

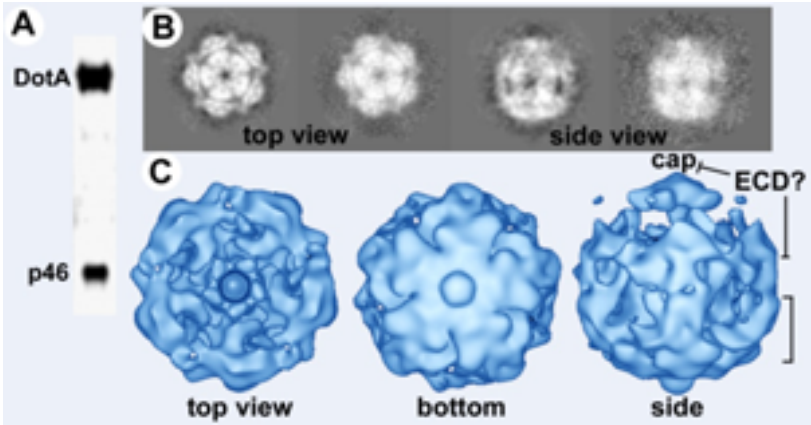
・Periplasmic component
IcmX　?

・Cytosolic components
DotB　ATPase
IcmS/IcmW　global chaperone?
IcmQ/IcmR　?

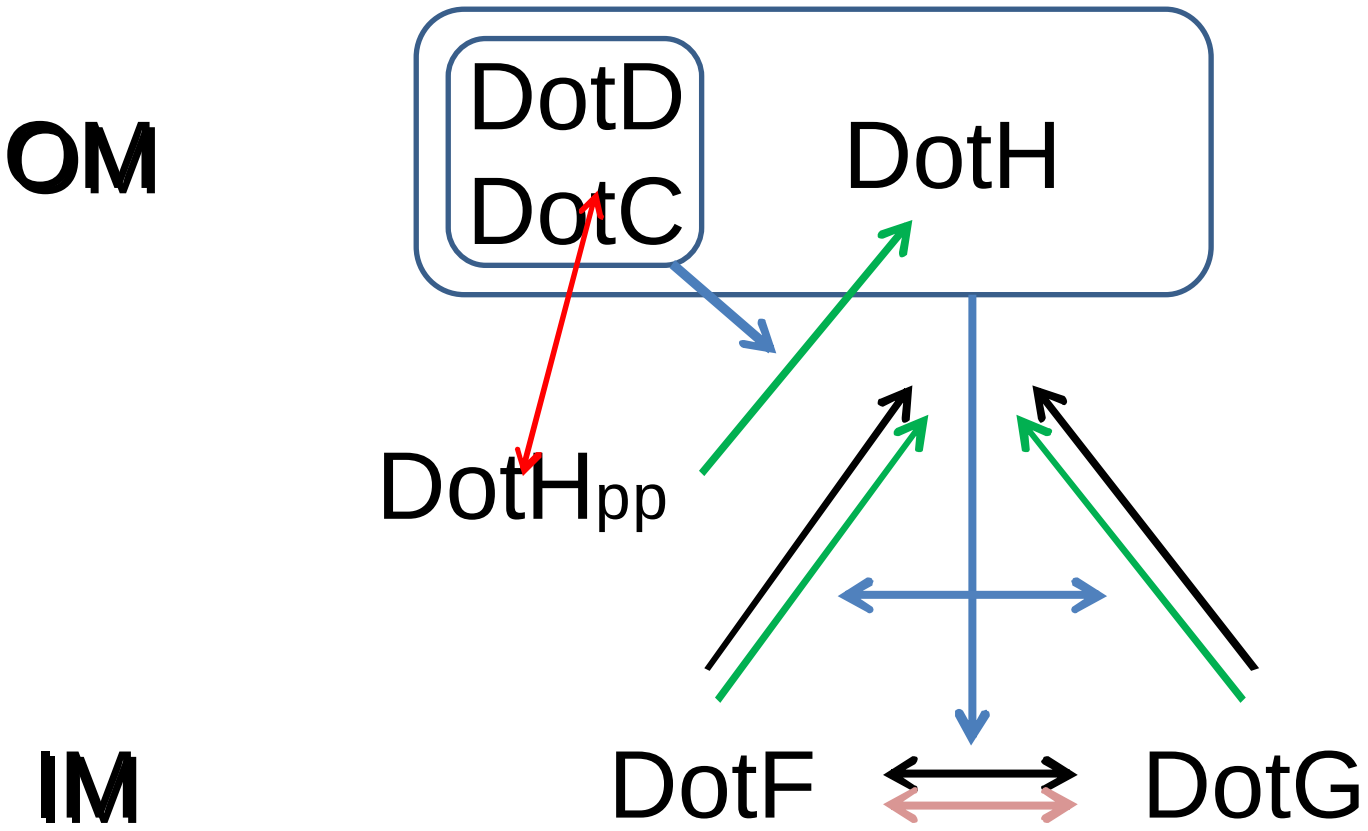
・Inner membrane / extracellular
DotA　forms ring-like structure



DotA (unpublished)



Lines of evidence suggest a complex containing DotC/DotD/DotF/DotG/DotH.

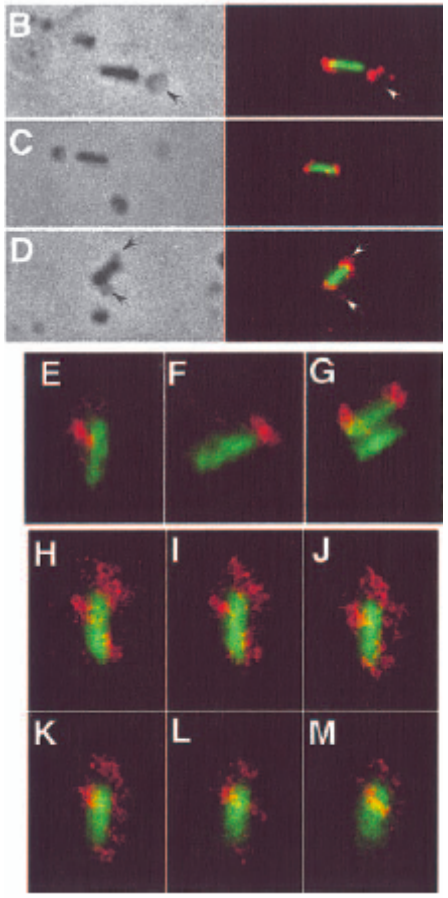


CoIP B2H Localization Functional analysis Biochemisry

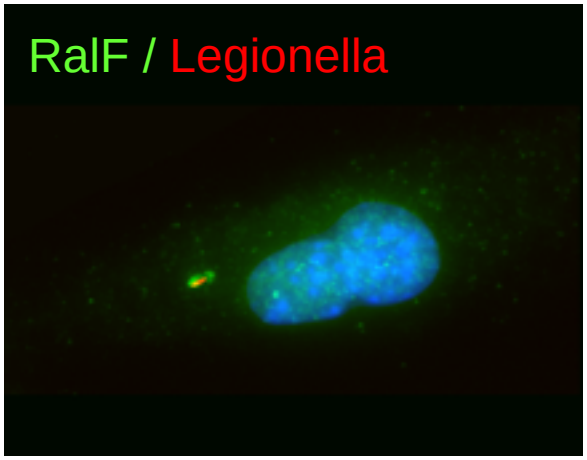
Does the Dot/Icm T4SS localize to cell poles?

Legionella effectors were found in the vicinity of the bacterial cell poles

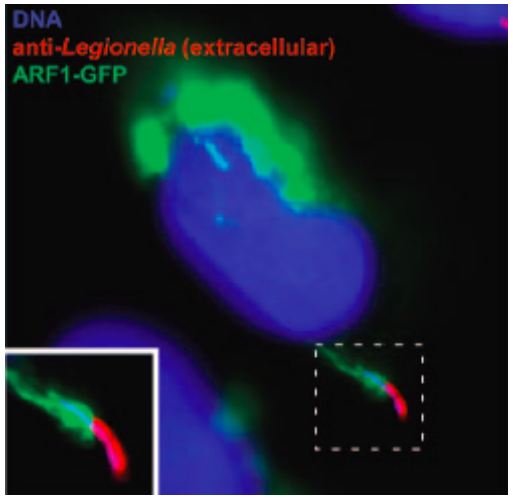
LidA on isolated *Legionella*-containing vacuole



Conover *et al*. *Mol. Microbiol.* 2003

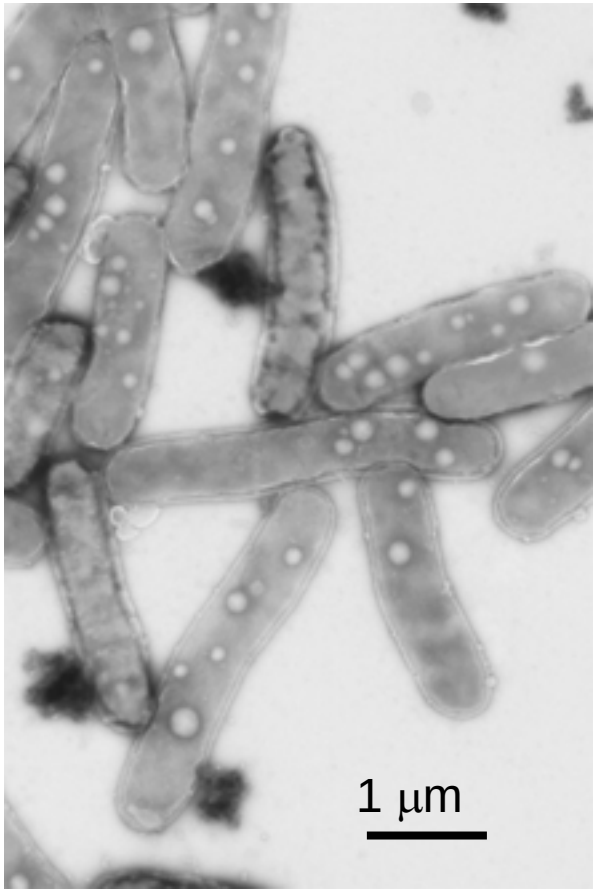


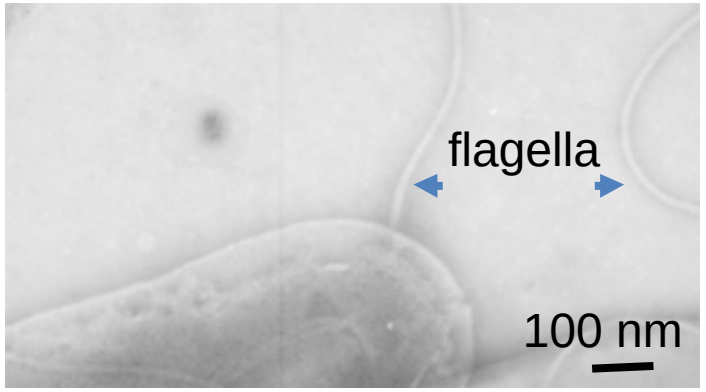
By H. Nagai
unpublished

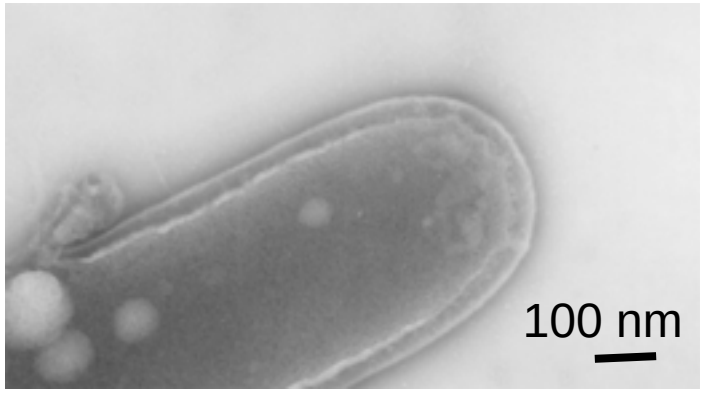


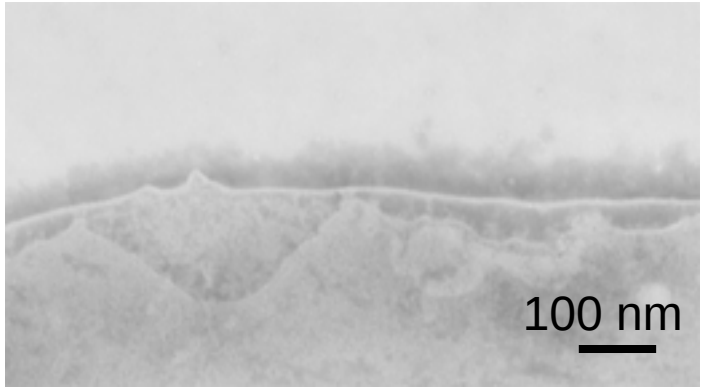
By Jon Kagan
Nagai *et al*. *PNAS* 2005

No needle-like structure was found on *L. pneumophila* cell surface

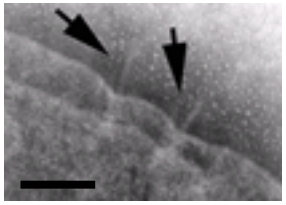


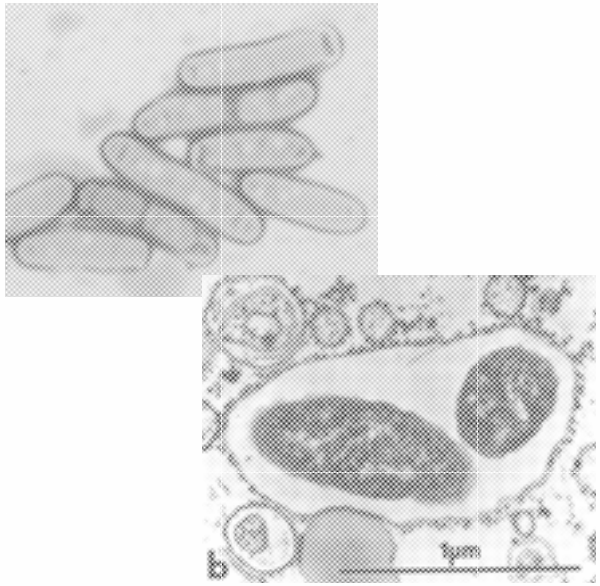






Type III
Needle complexes





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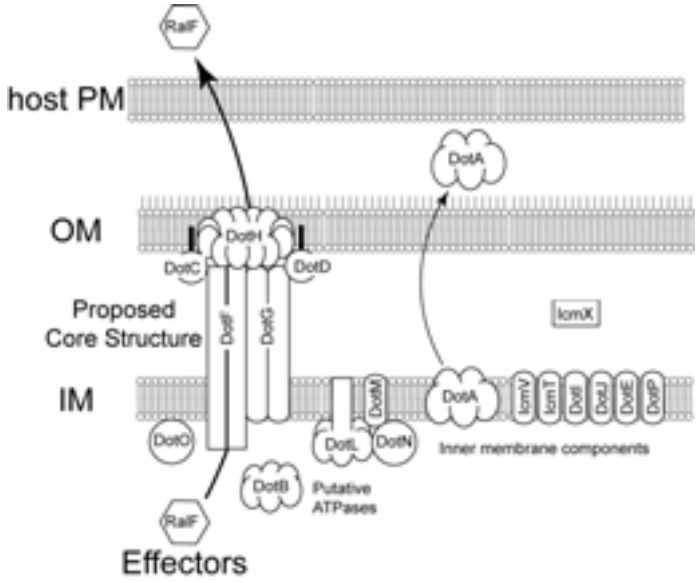
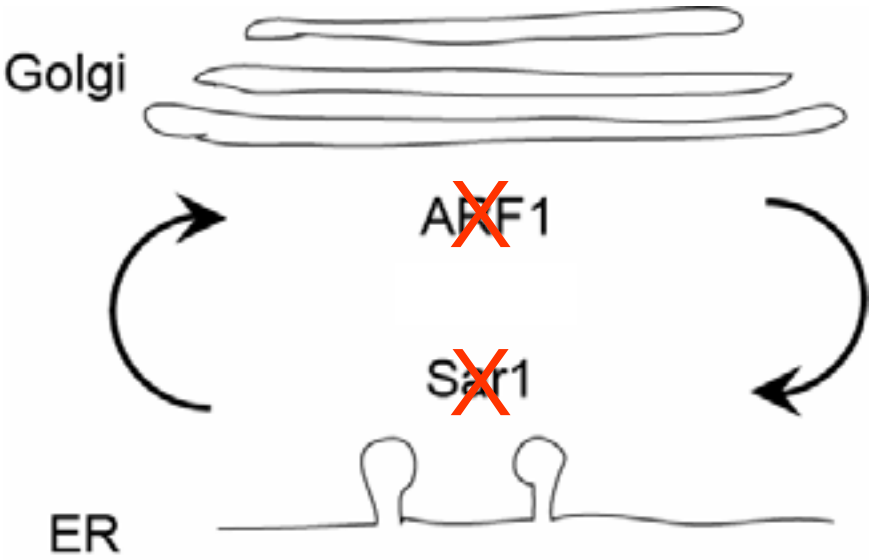
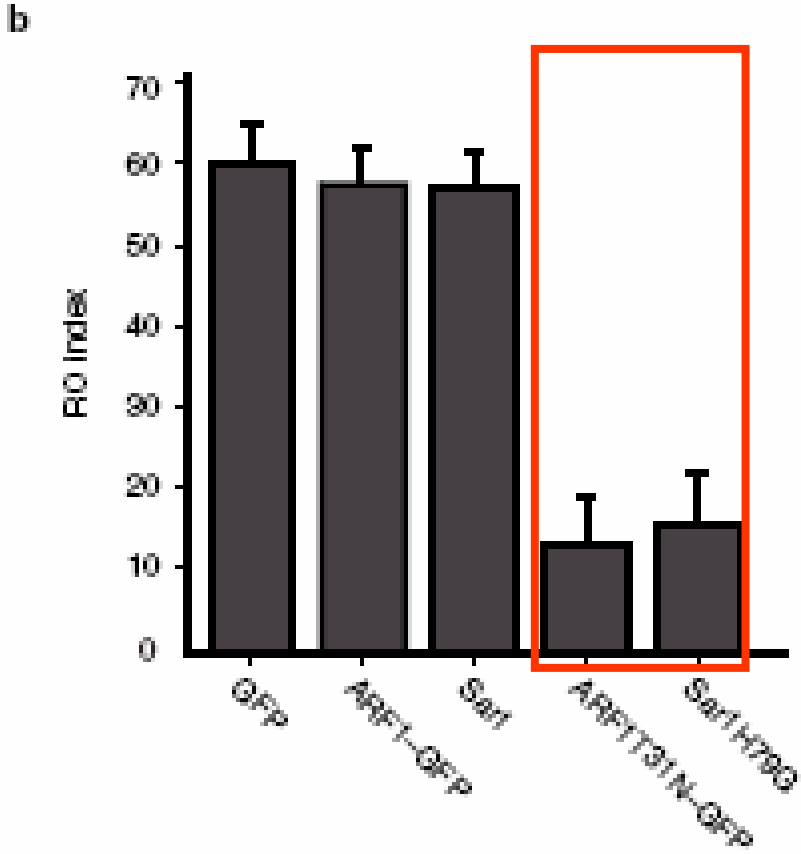


Inhibition of Sar1 or ARF1 by d/n alleles, by knock-down or by ARF inhibitor BFA severely restrict intracellular growth of *Legionella*



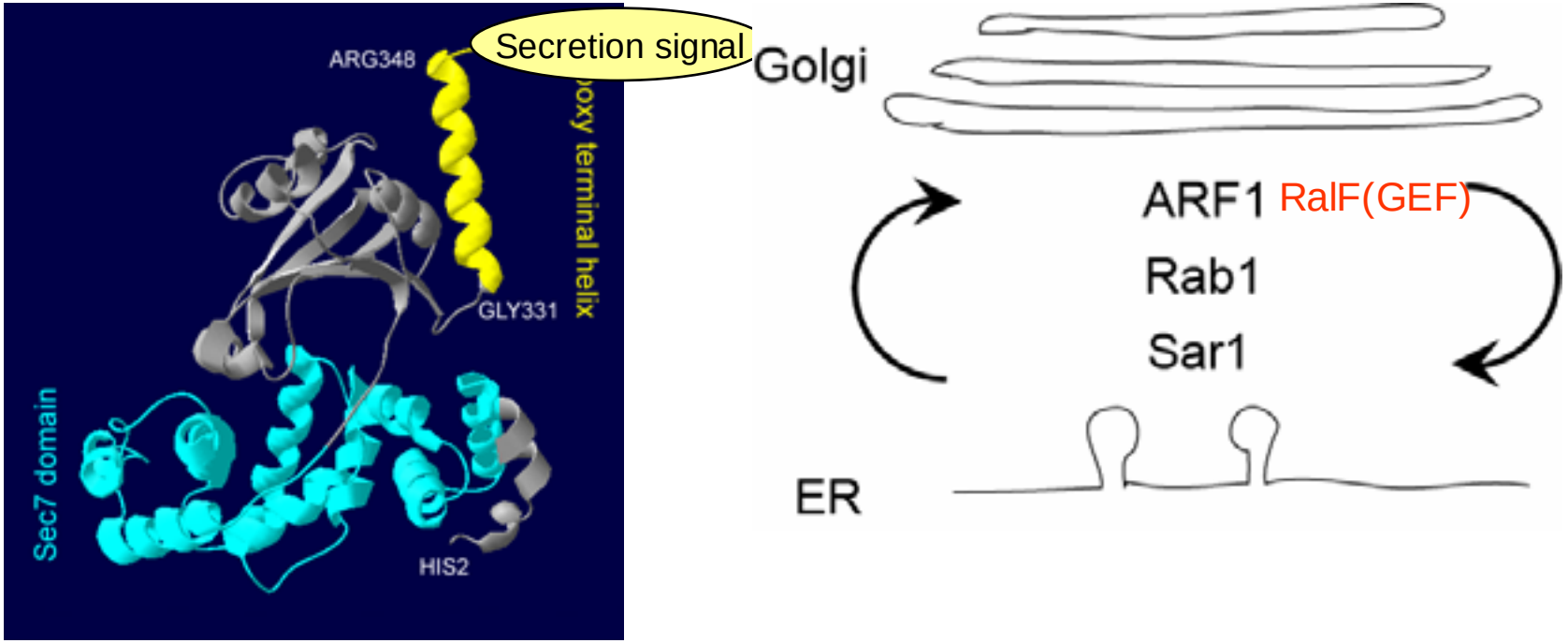


Jon Kagan and Craig Roy
Nat. Cell Biol. 2002

Legionella effector proteins and membrane traffic between ER-Golgi

RalF

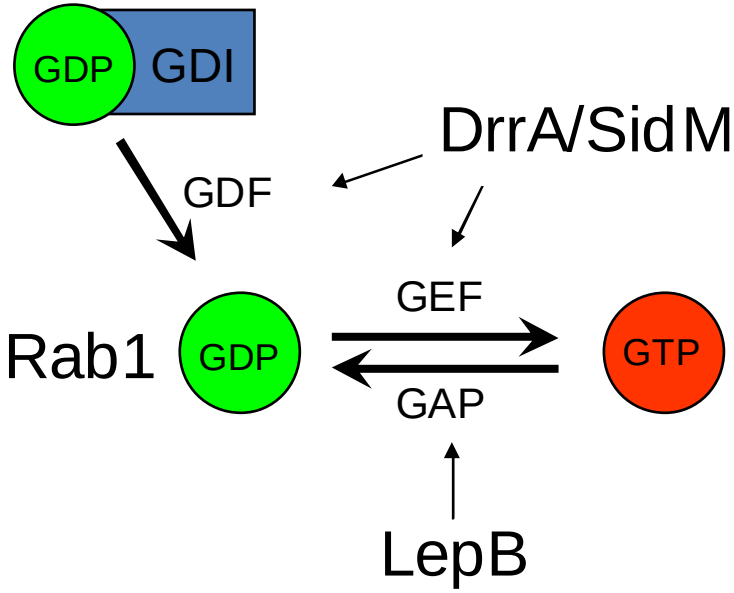
GEF for ARF1

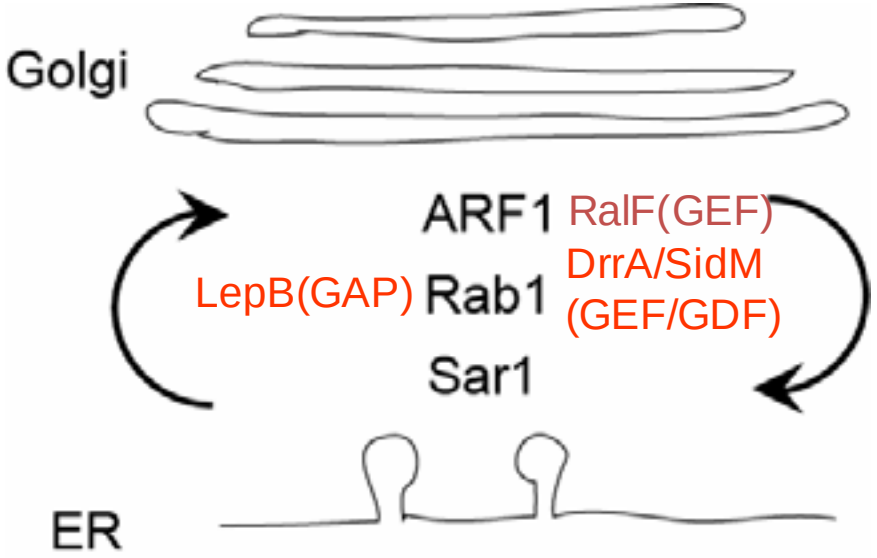


Nagai et al. Science 2002
Amor et al. J. Biol. Chem. 2005
Nagai et al. PNAS 2005

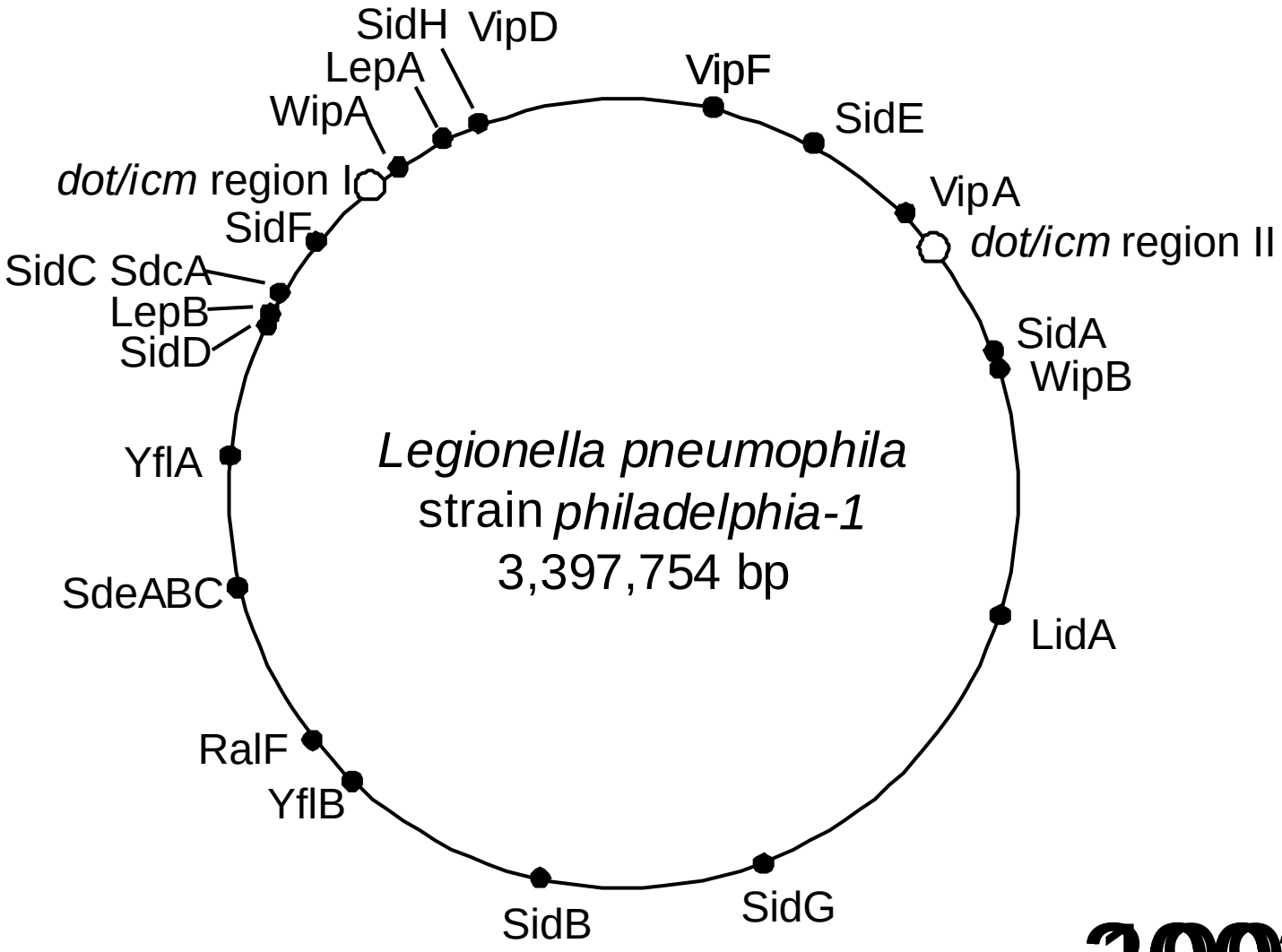
Legionella effector proteins and membrane traffick between ER-Golgi

DrrA/SidM and LepB modulate Rab1 activity.





Murata et al. Nat. Cell Biol. 2006
Machner et al. Dev. Cell 2006
Ingmundson et al. Nature 2007
Machner et al. Science 2007

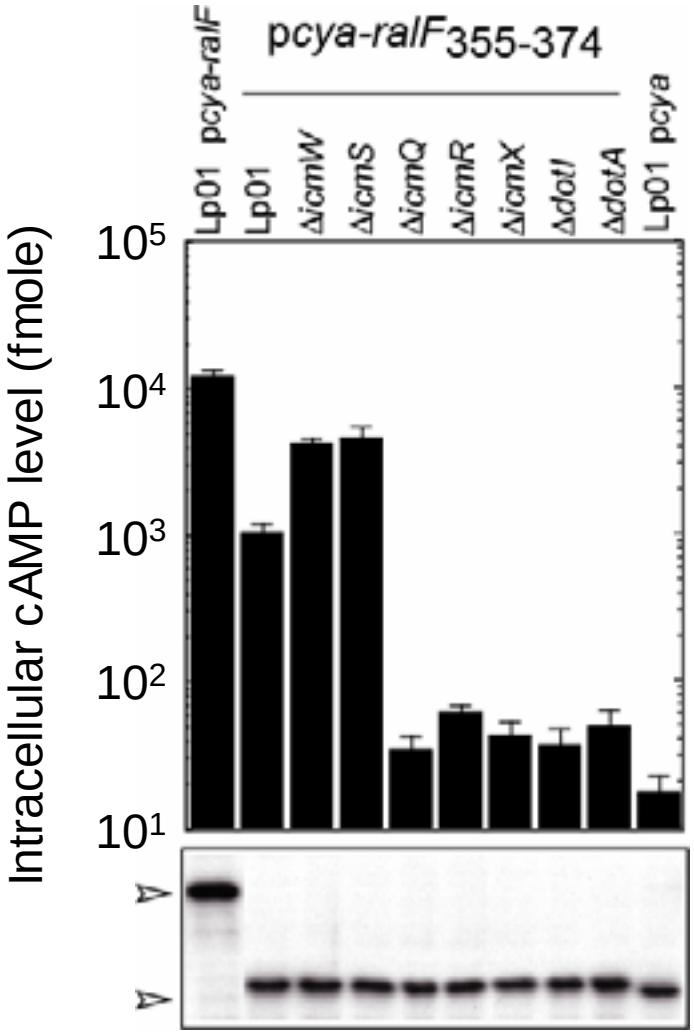

SidH
VipD
LepA
VipF
WipA
SidE
dot/icm region I
VipA
SidF
dot/icm region II
SidC
SdcA
LepB
SidA
SidD
WipB
YflA
Legionella pneumophila
strain philadelphia-1
3,397,754 bp
SdeABC
LidA
RalF
YflB
SidB
SidG
~2090
~2990

Towards high-throughput systematic screening of effector proteins.

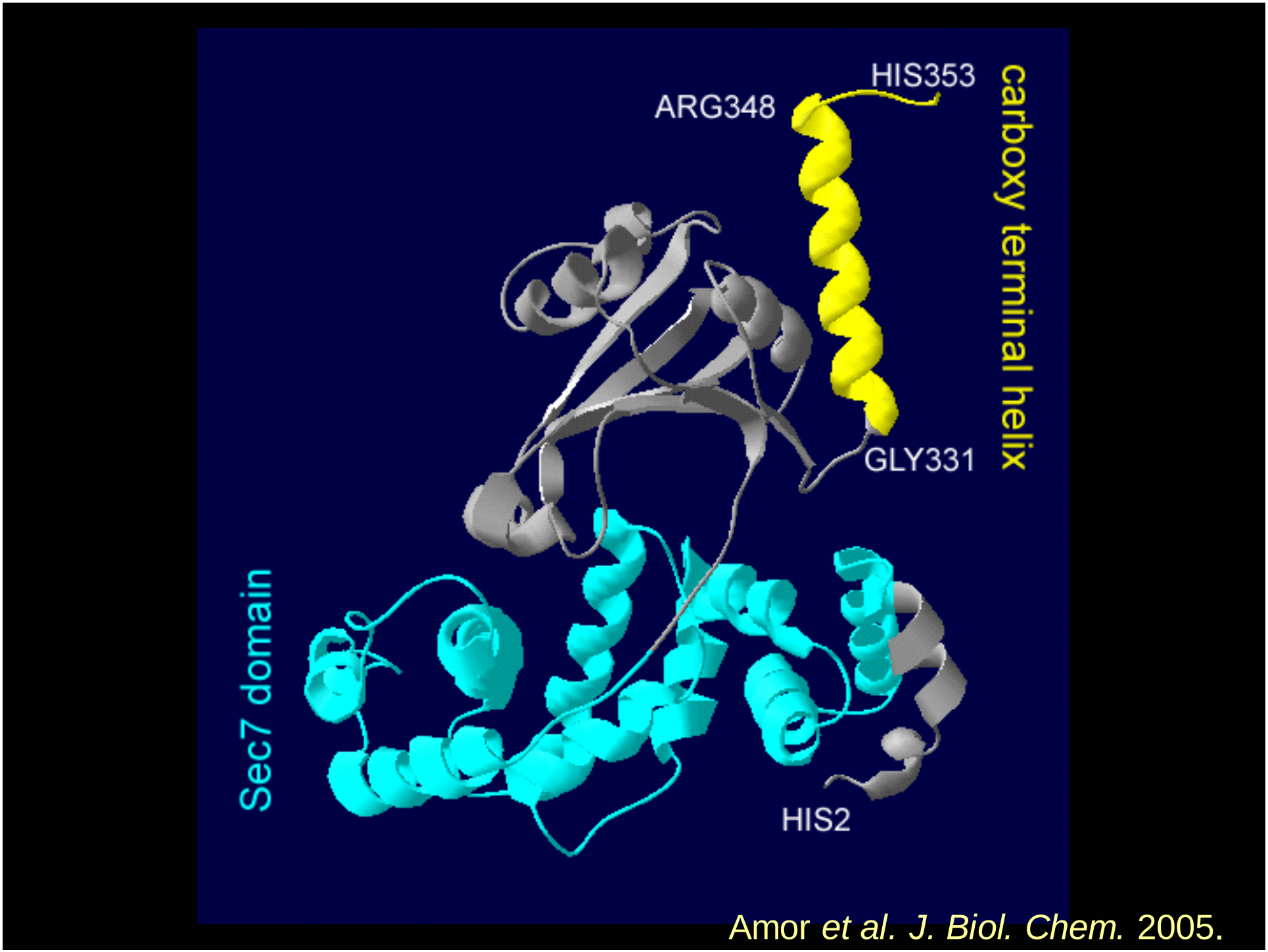
- Establish a reporter system to detect protein translocation to host cell cytoplasm.
  - Unavailability of such a system was a bottle-neck of the *Legionella* research.

- Analyses of secretion signals of known effector proteins.
  - Together with genome sequence completed 2004, the outcome will help *in silico* screening of putative effector proteins.

Carboxy terminal 20 amino acid region of RalF is sufficient for translocation by the Dot/Icm TFSS.



Nagai *et al. PNAS* 2005.



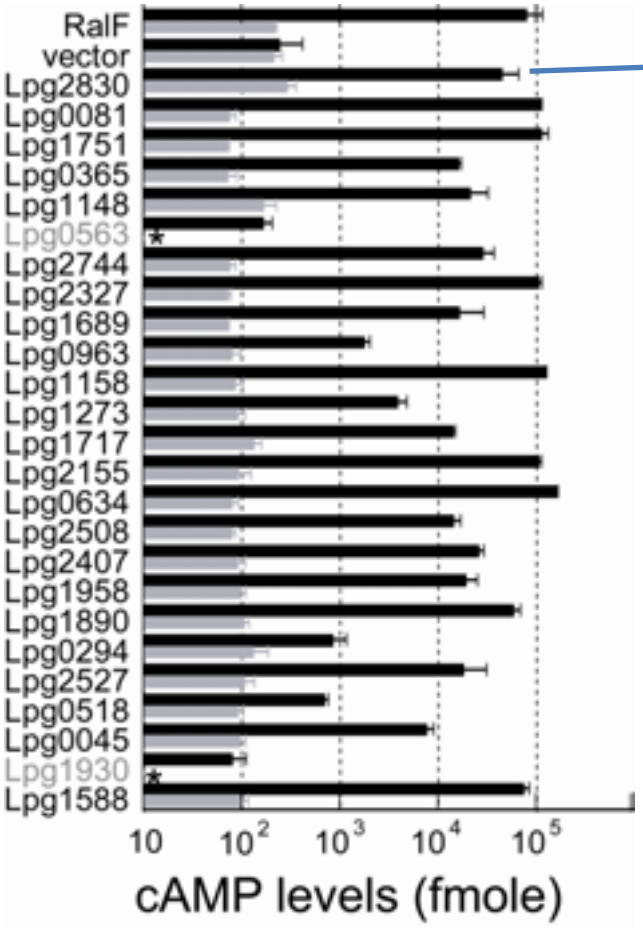
Amor *et al. J. Biol. Chem.* 2005.

Hydrophobicity of the third last residue appeared to be important for RalF translocation by the *Legionella* Dot/Icm secretion system.

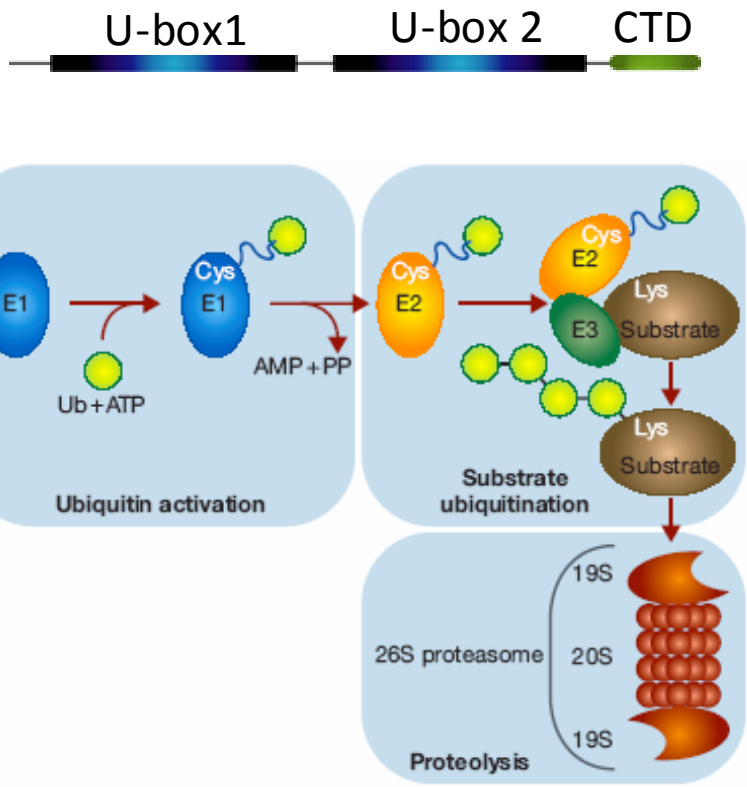
		% Translocation
RalF	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**KF	100
1-373	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**K	24
1-372	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**	0.5
1-371	TIERNLALKEGVPKDPDAEMQKEKGRQ	0
L372F	TIERNLALKEGVPKDPDAEMQKEKGRQ**F**KF	99
L372P	TIERNLALKEGVPKDPDAEMQKEKGRQ**P**KF	89
L372V	TIERNLALKEGVPKDPDAEMQKEKGRQ**V**KF	20
L372A	TIERNLALKEGVPKDPDAEMQKEKGRQ**A**KF	18
L372S	TIERNLALKEGVPKDPDAEMQKEKGRQSKF	2.7
L372T	TIERNLALKEGVPKDPDAEMQKEKGRQTKF	0.3
K373A	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**AF	81
K373E	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**EF	93
K373R	TIERNLALKEGVPKDPDAEMQKEKGRQ**L**RF	125

Nagai *et al. PNAS* 2005.

Dot/Icm substrate proteins identified in our lab.



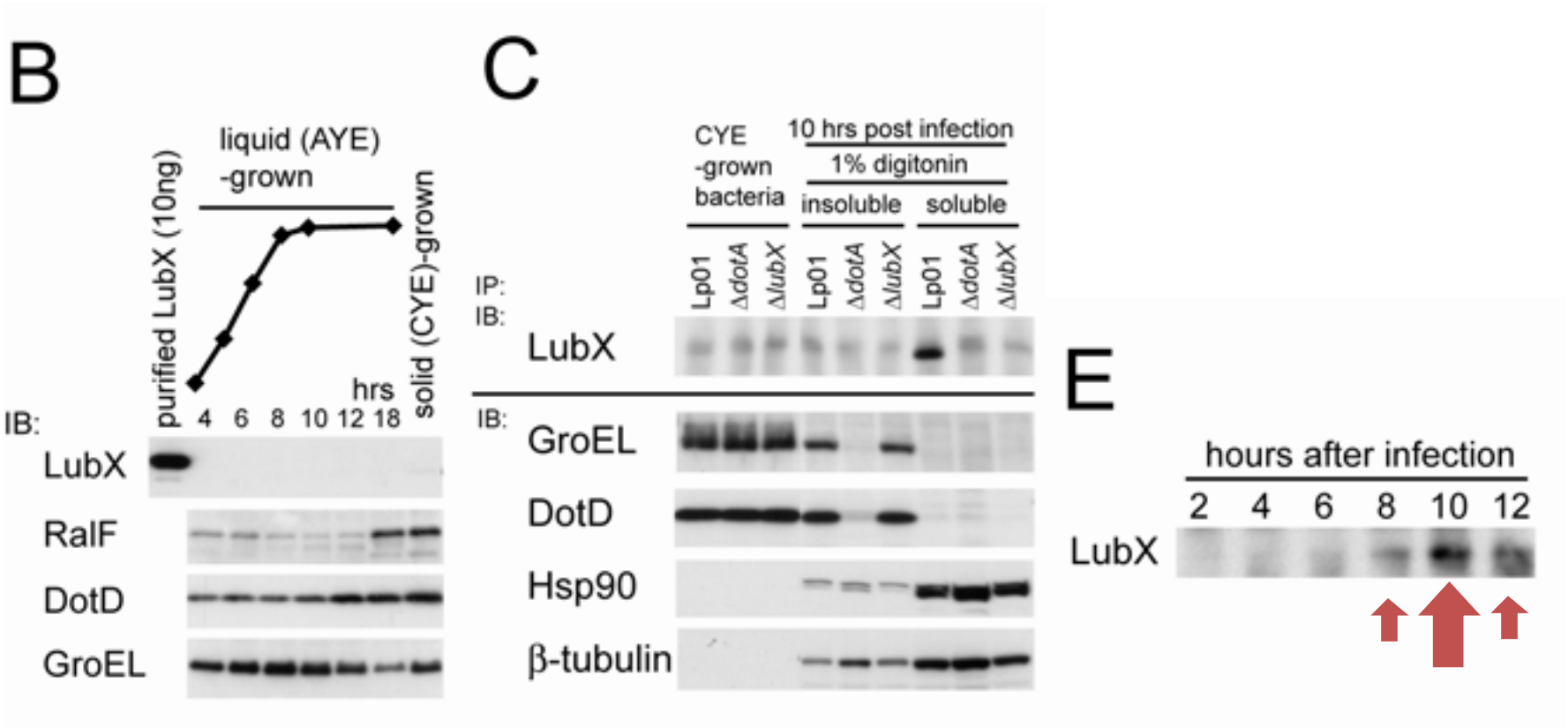
LubX:
Legionella U-box-containing protein



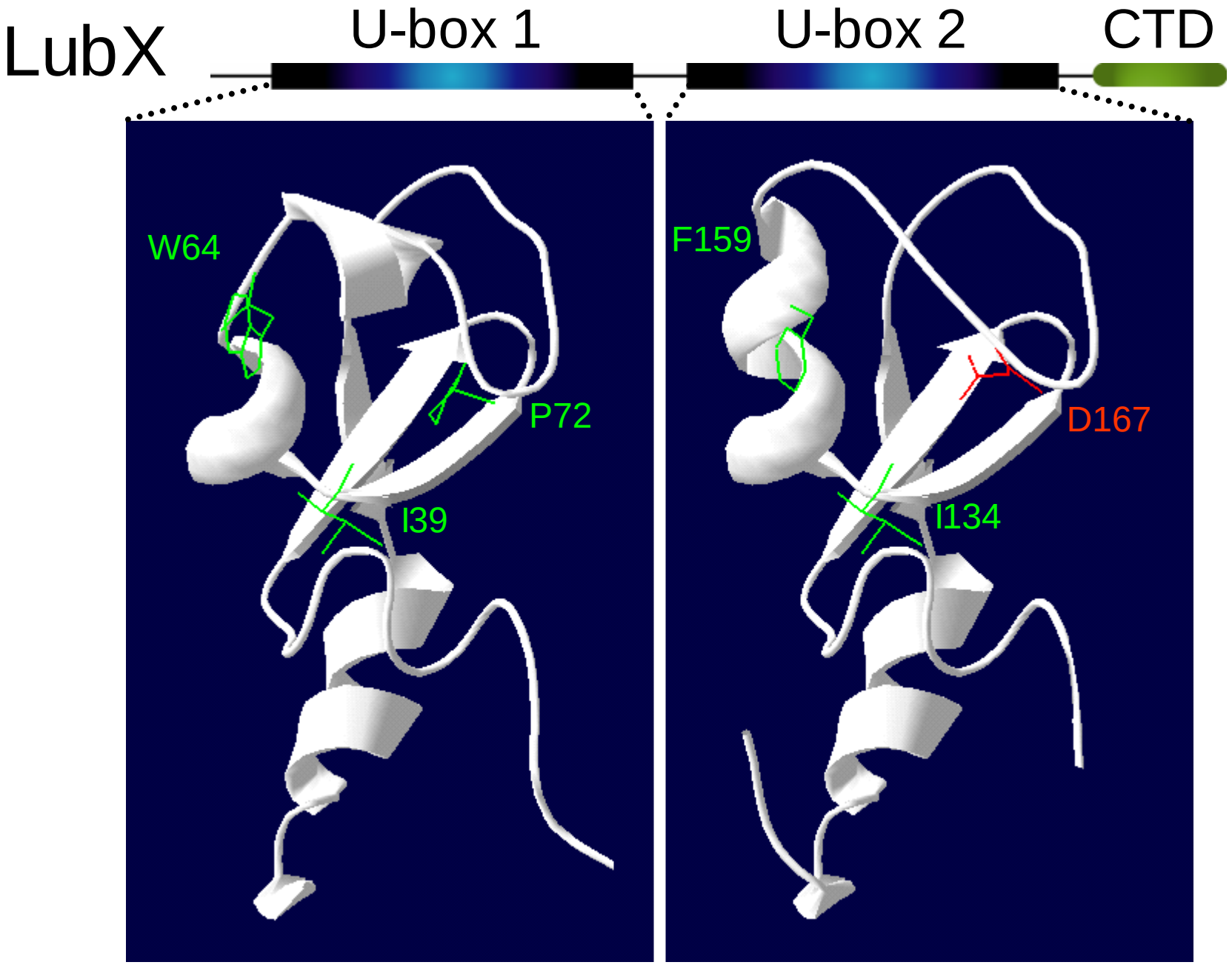
Question:
Is LubX an E3 ubiquitin ligase?

Kubori et al. *Mol Microbiol* 2008

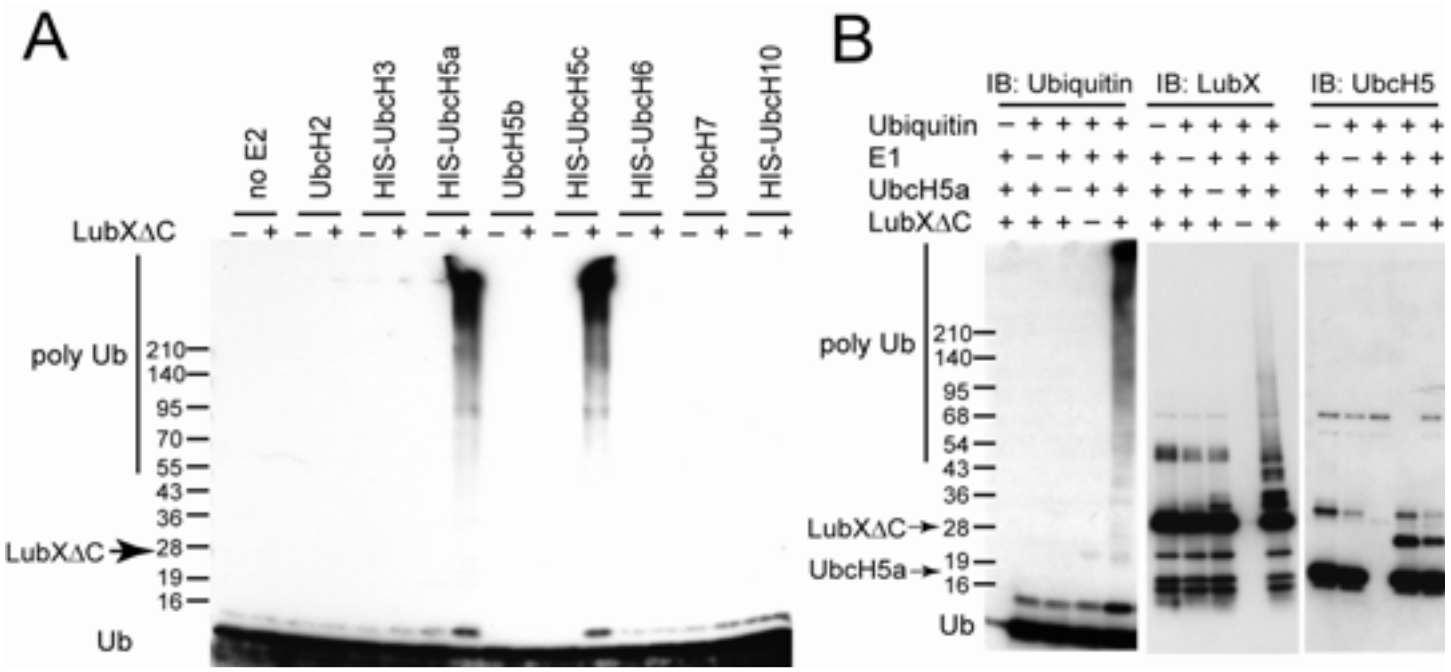
Expression of LubX is induced upon infection, and LubX level within the host cells peaks at a late stage of infection.



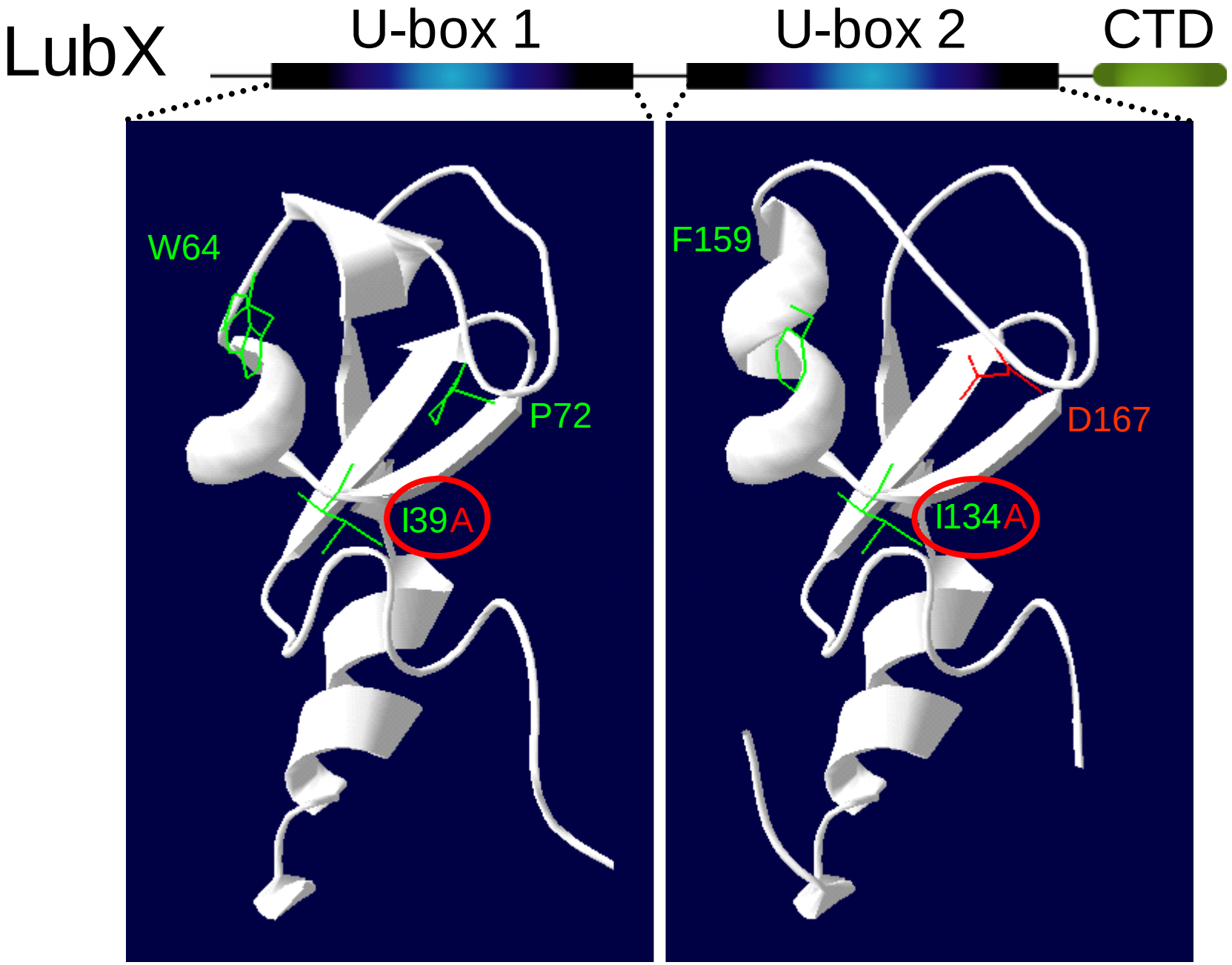
Kubori *et al. Mol. Microbiol.* 2008.


LubX
U-box 1
U-box 2
CTD
W64
P72
I39
F159
D167
I134

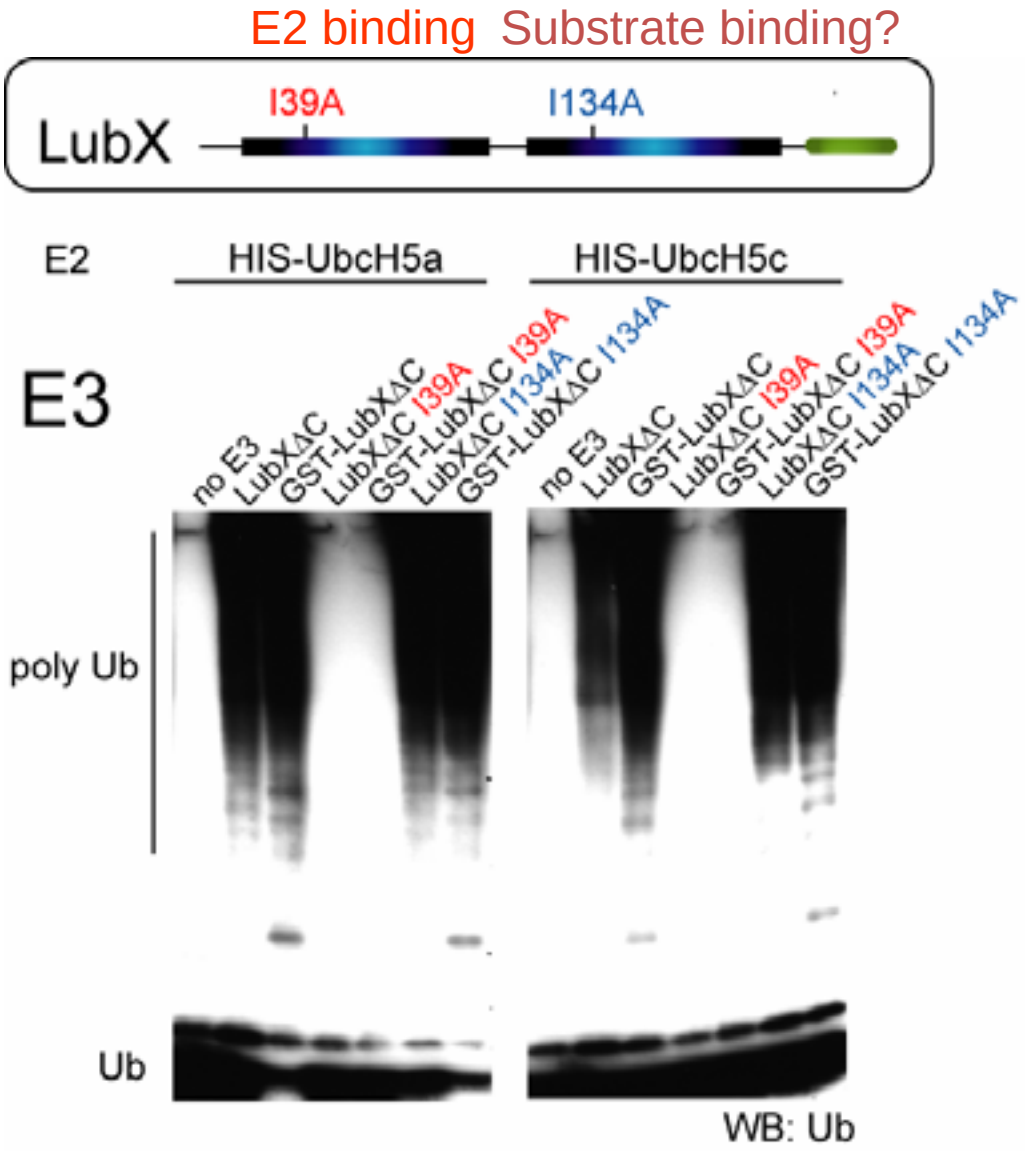
LubX functions as an E3 Ubiquitin ligase in conjunction with the E2 enzymes UbcH5a or UbcH5c.



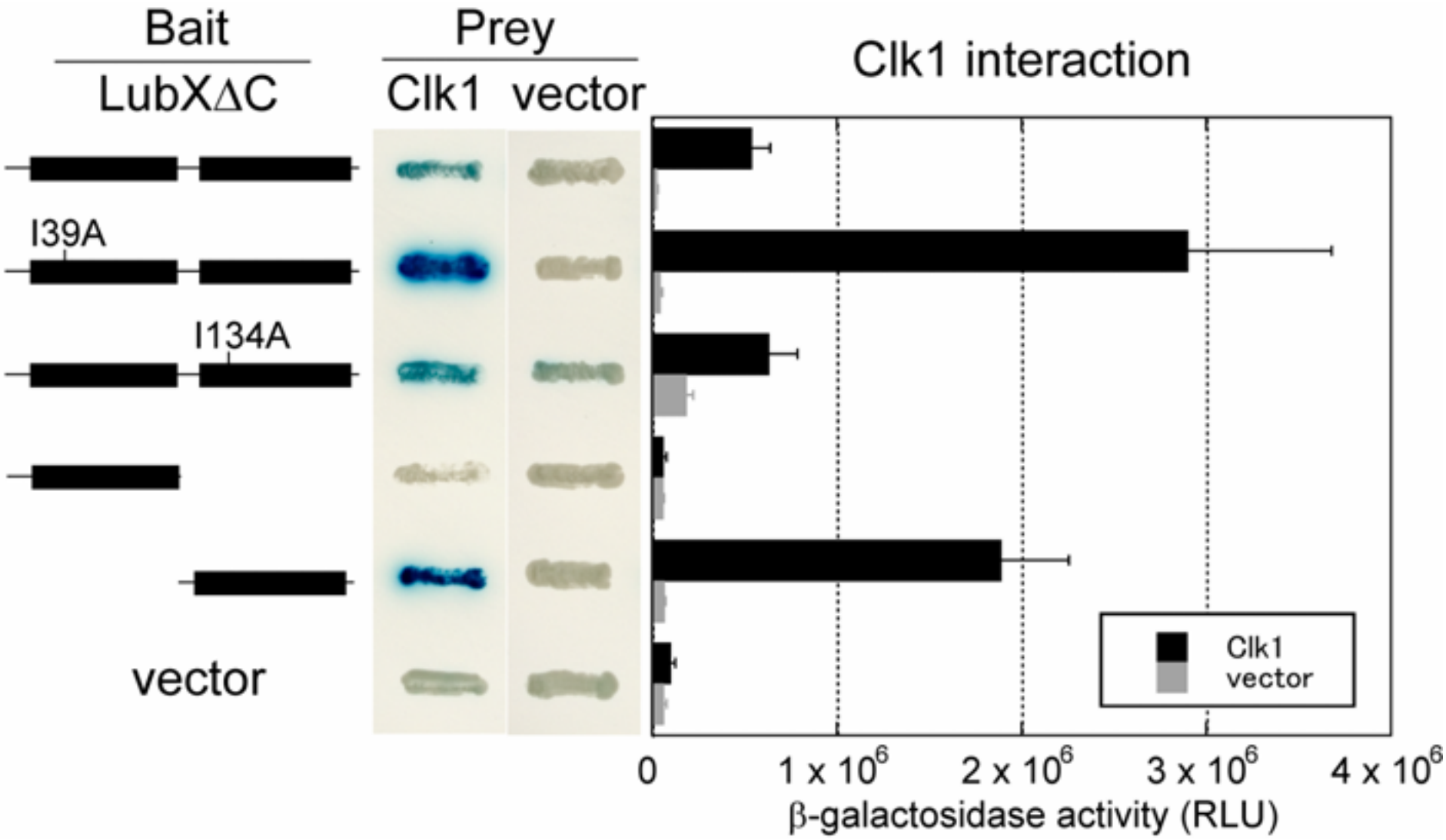
Kubori et al. *Mol Microbiol* 2008


LubX
U-box 1
U-box 2
CTD
W64
P72
I39A
F159
D167
I134A

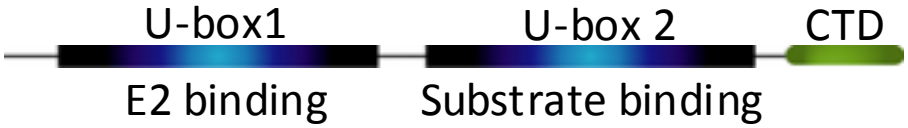
U-box 1 is essential to E3 Ubiquitin ligase activity.



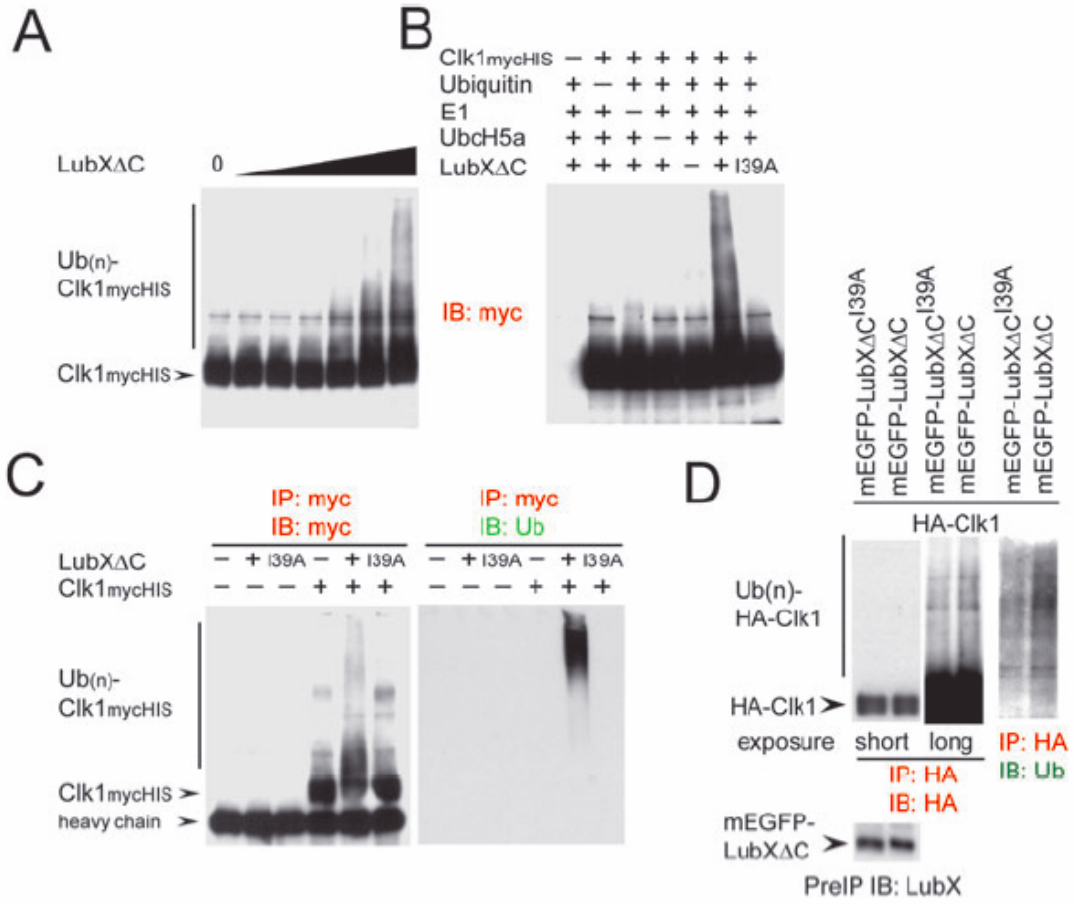
Two hybrid interaction of Clk1 and the LubX U-box 2.



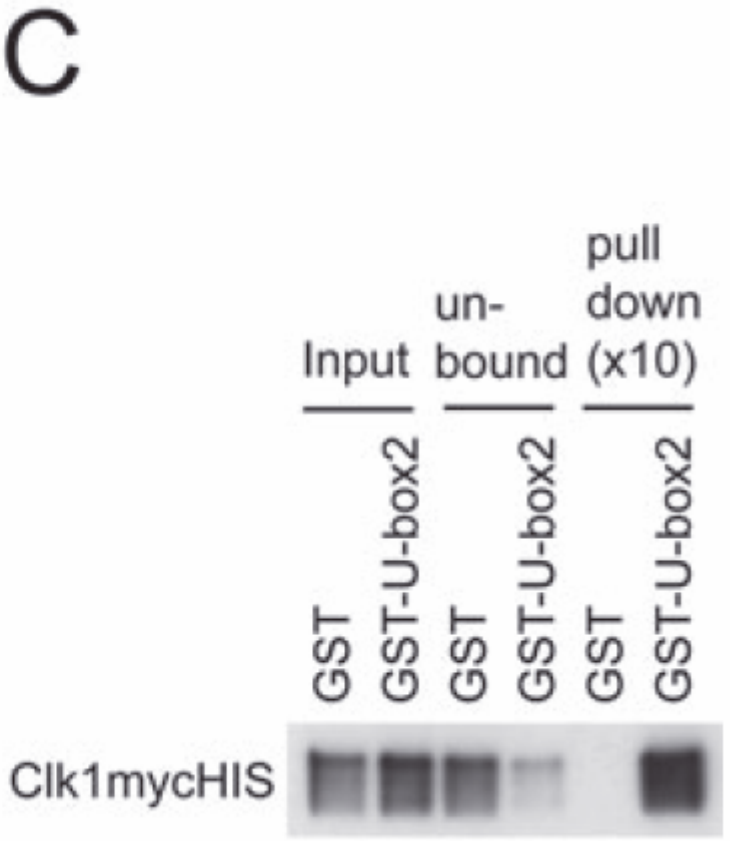
LubX U-box 2 functions as a substrate binding site.



Clk1 is a substrate of LubX



Direct interaction of Ubox2 and Clk1



Kubori *et al. Mol. Microbiol.* 2008.