Recherche fondamentale en Maladies Infectieuses: exemple de *Legionella*

Séminaire DESC

Pathologie Infectieuse et Tropicale

2010-2011

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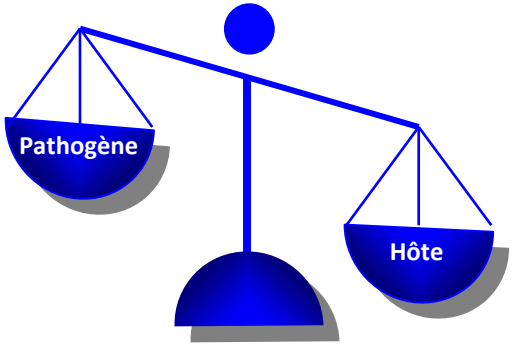
2 aspects:

- qu'est ce que la recherche fondamentale sur une bactérie pathogène comme *Legionella* apporte à la connaissance que l'on a de la relation hôte-pathogène?

- pour un(e) infectiologue : de combien de connaissances scientifiques fondamentales a-t-il(elle) besoin et quel est l'intérêt de faire de la recherche fondamentale ?

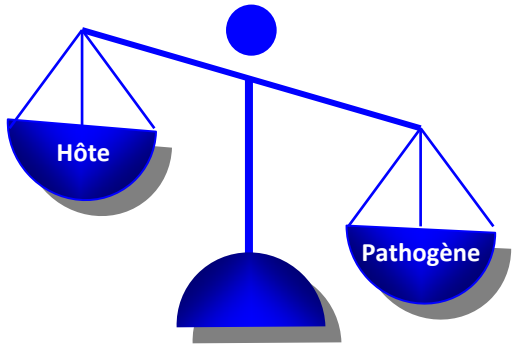
Modèle en résistance



Décryptage des mécanismes de résistance développés par l'hôte

- Clairance
  - Mémoire Immunitaire ?
- Tolérance
  - Quiescence/réactivation colonisation

Modèle en virulence



Identification des facteurs et mécanismes de virulence bactérienne à l'origine de la défaillance de l'hôte

Cas particulier des bactéries intracellulaires

Dans le cadre des maladies vectorielles, la coévolution au sein des réservoirs environnementaux conditionne la virulence vis-à-vis de l'hôte humain

↓

Transition adaptative

\- ----------→ +

Protozoaires unicellulaires eucaryotes

↓

Bio cell : exploitation des fonctions élémentaires
Immuno: fitness faible

↓

Legionella

Mammifères vertébrés
Réservoir animal

↓

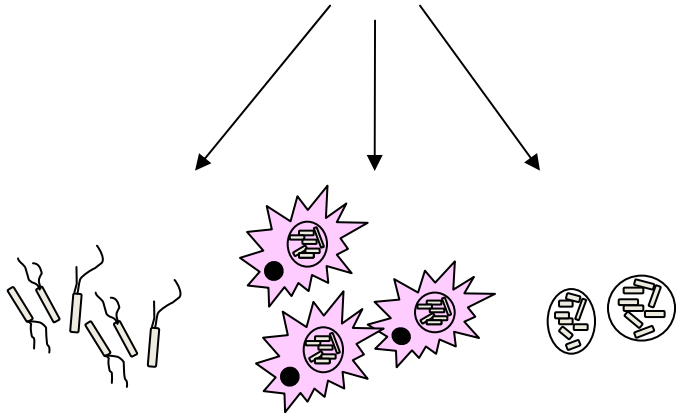
Bio cell : exploitation des fonctions élémentaires et complexes
Immuno: fitness optimisé, chronicité
± Transmission interhumaine

↓

Mycobacterium, Coxiella

Freshwater source
Cooling towers
Protozoan organisms

Water droplets



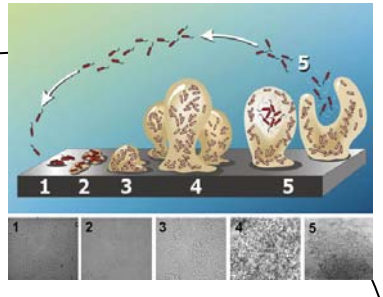
Human lungs
Alveolar macrophages
Legionnaires' disease

1ère partie: la dynamique au sein des réservoirs environnementaux
Du biotope aquatique à l'homme

Naturel

Domestiqué

Habitat dulçaquicole



Biofilms

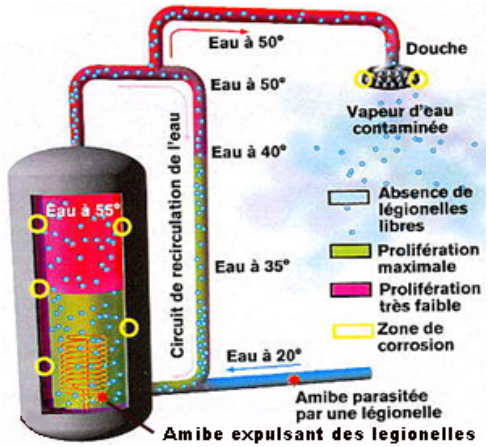
Protozoaires eucaryotes (amoeba)

Vacuoles

Libres planctoniques

Tartre

Tours aeroréfrigérantes
Installations collectives



Cumulus
Installation individuelle

Risque individuel (cas sporadique) ↔ Risque collectif (cas épidémiques)

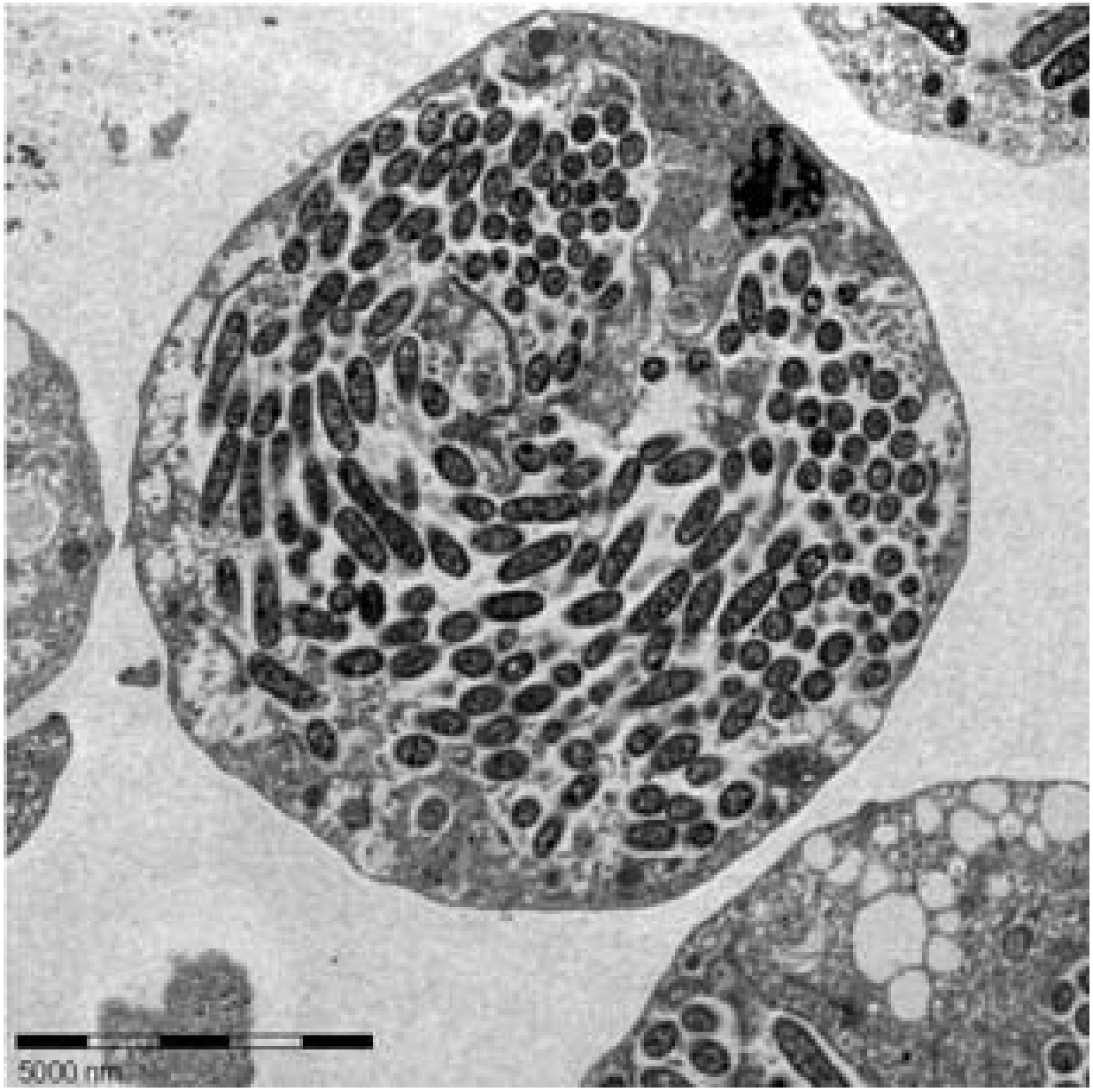
Risque individuel (cas sporadique) ↕ Risque communautaire

Risque collectif (cas épidémiques) ↕ Risque nosocomial

Risque individuel (cas sporadique) ↔ Risque nosocomial

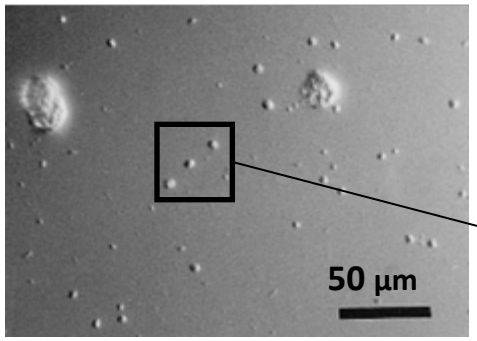
Risque collectif (cas épidémiques) ↔ Risque communautaire

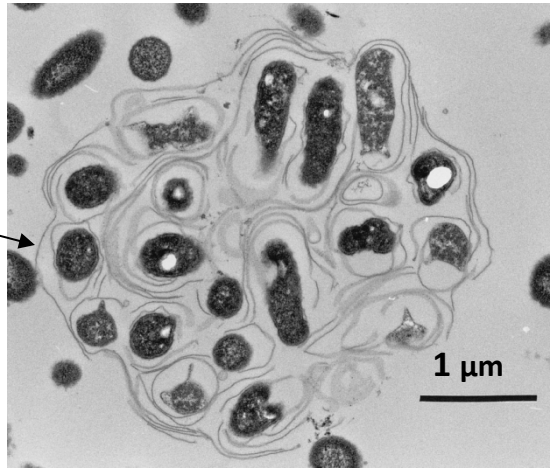
«Risque légionelle» : seuil d'aérocontamination NON prévisible

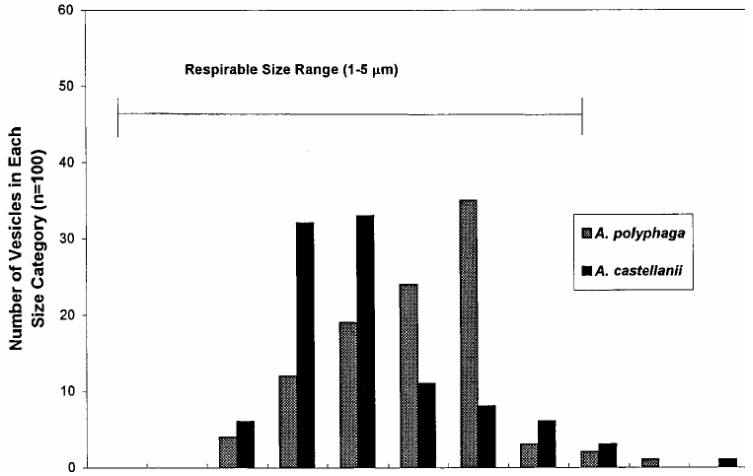

5000 nm

Higher occurrence of infected amoebae in cooling towers compared with natural aquatic environments

Acanthamaoba spp. can expell *Legionella*-containing vacuole (LCV) in condition of stress (temperature, biocides) prior to encystment







Berk et al. 1998

3 characteristics:
-free in the medium
-number of bacteria /LCV: 20 ↔ 1483
-resistance^{++} (temperature, biocides)

2ème partie: de la biologie cellulaire eucaryote élémentaire à la biologie cellulaire mammifère humaine

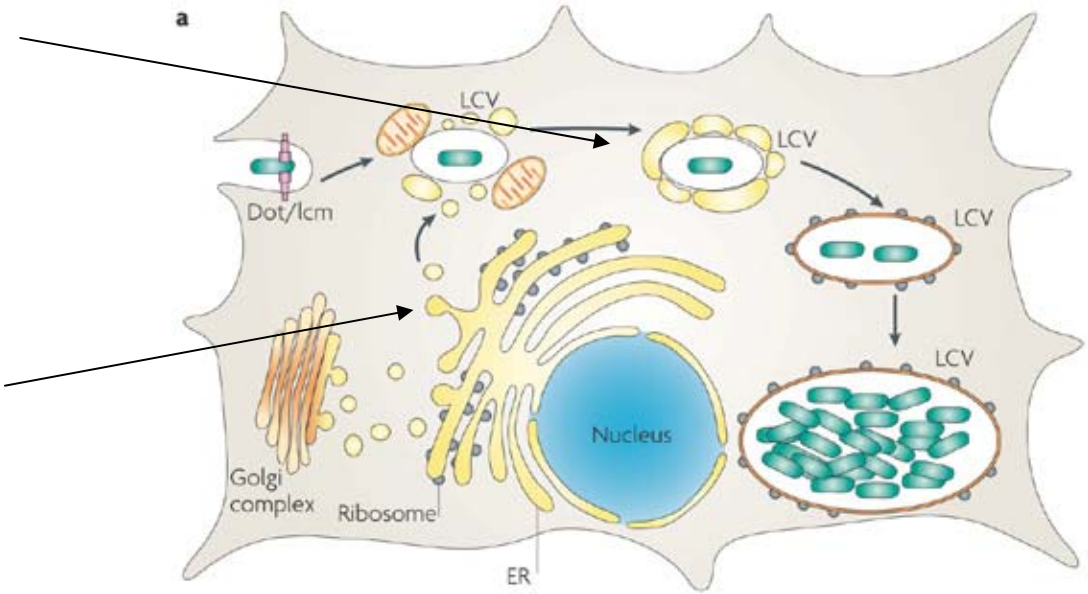
Stratégie:
utiliser les voies de trafficking de la cellule hôte à son profit → réplication

Moyens :
-posséder un appareil conjugatif capable de « transloquer » des protéines substrats dans le cytosol → Appareil de sécrétion de type IV (Type IV secretion system) **= Dot/Icm**
-posséder un répertoire de protéines substrats « eukaryotic-like » qui prennent contrôle des voies de trafficking intracellulaire

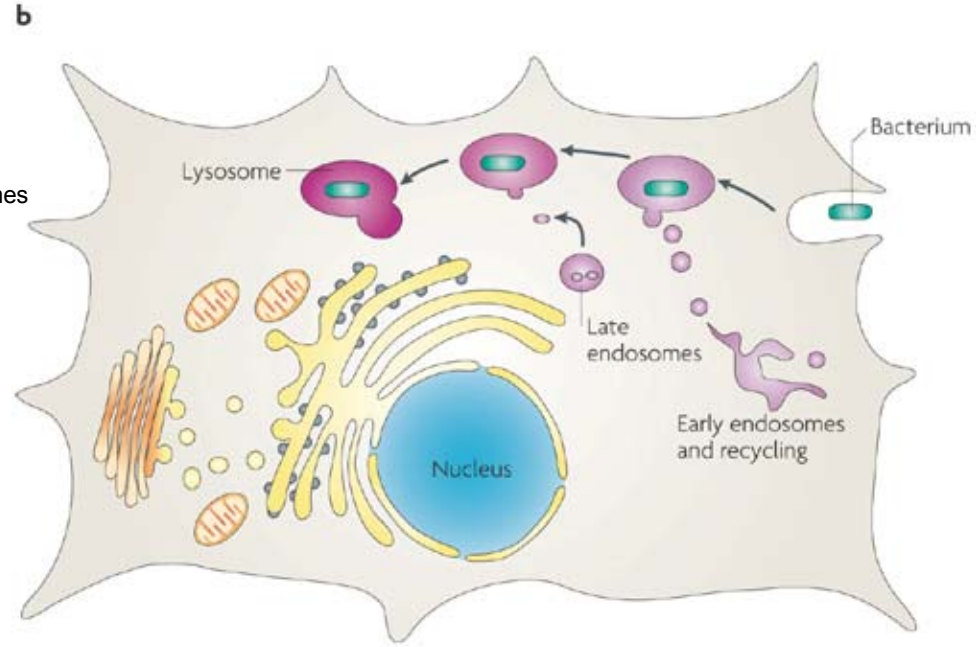
Cible :
Cellules phagocytaires type macrophages alvéolaires > cellules épithéliales

Horwitz, M. A. Formation of a novel phagosome by the Legionnaires' disease bacterium (*Legionella pneumophila*) in human monocytes. J. Exp. Med. 158, 1319–1331 (1983).
This early study showed that *L. pneumophila* replicates inside eukaryotic cells within a morphologically distinct membrane-bound vacuole that evades fusion with endocytic compartments.

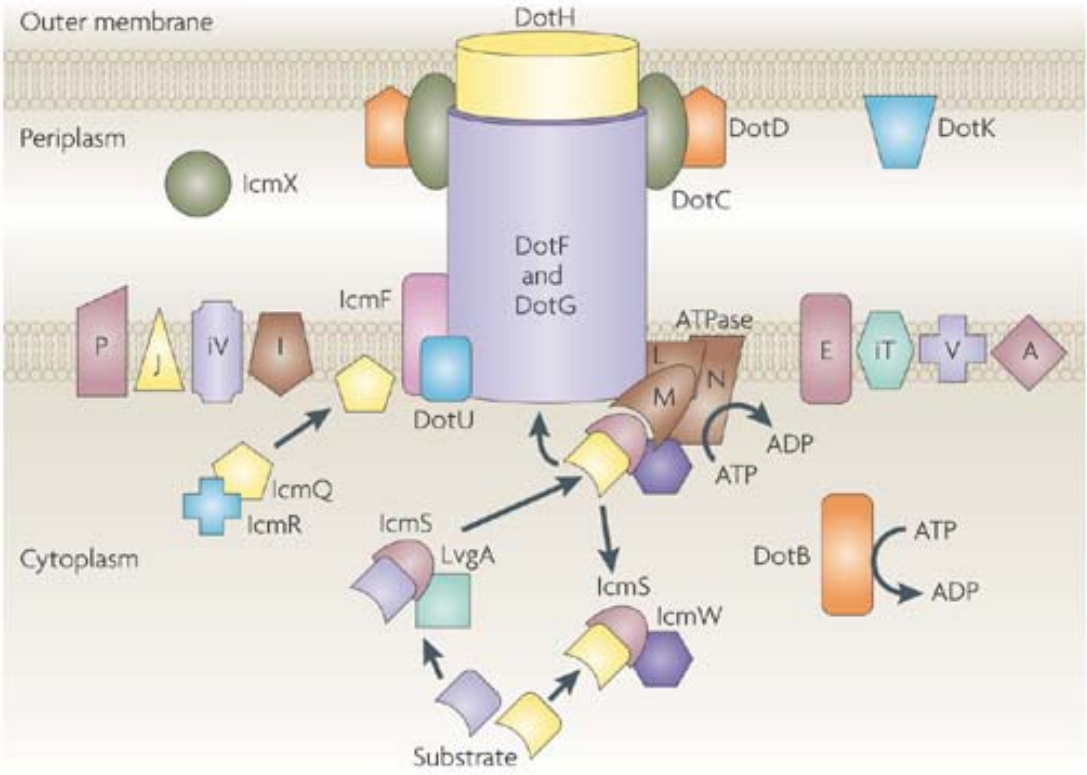
Swanson, M. S. & Isberg, R. R. Association of *Legionella pneumophila* with the macrophage endoplasmic reticulum. Infect. Immun. 63, 3609–3620 (1995).
Electron-microscopy based study which provides evidence that the LCV is similar to the host cell ER in morphology and protein composition.



Voie endosomo-lysosomiale:
voie de dégradation normale des microorganismes non pathogènes arrivant dans le cytosol.



Vincent, C. D. *et al*.
Identification of the core transmembrane complex of the *Legionella* Dot/Icm type IV secretion system. Mol. Microbiol. 62, 1278–1291 (2006).
Thorough study that detected distinct subcomplexes in the Dot/Icm translocon.

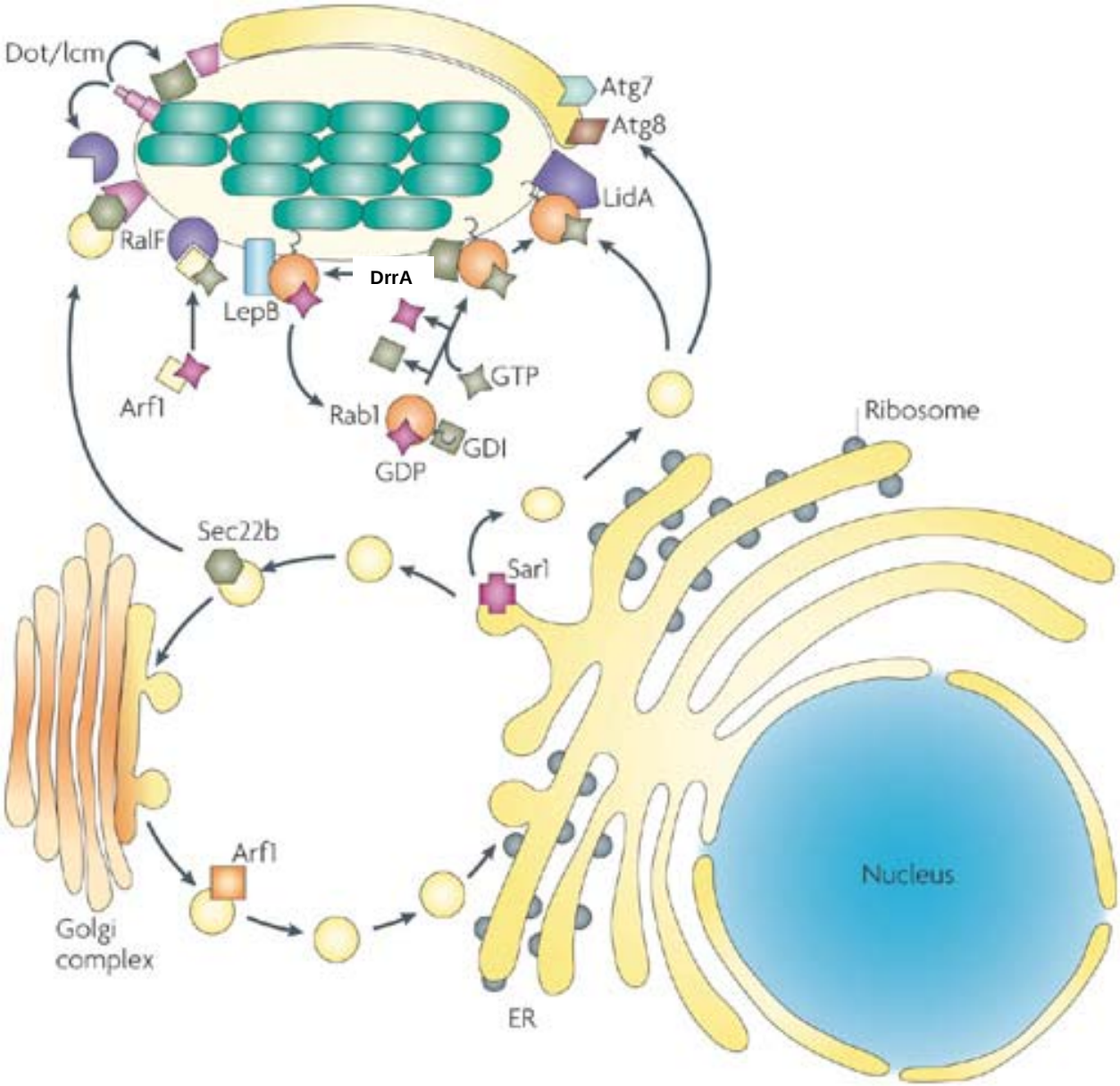


Nature Reviews | Microbiology

Vogel, J. P. & Isberg, R. R.
Conjugative transfer by the virulence system of *Legionella pneumophila*. Science 279, 873–876 (1998).
Provides evidence that the *dot/icm* genes encode a secretion system that can transfer plasmid DNA between bacterial cells.

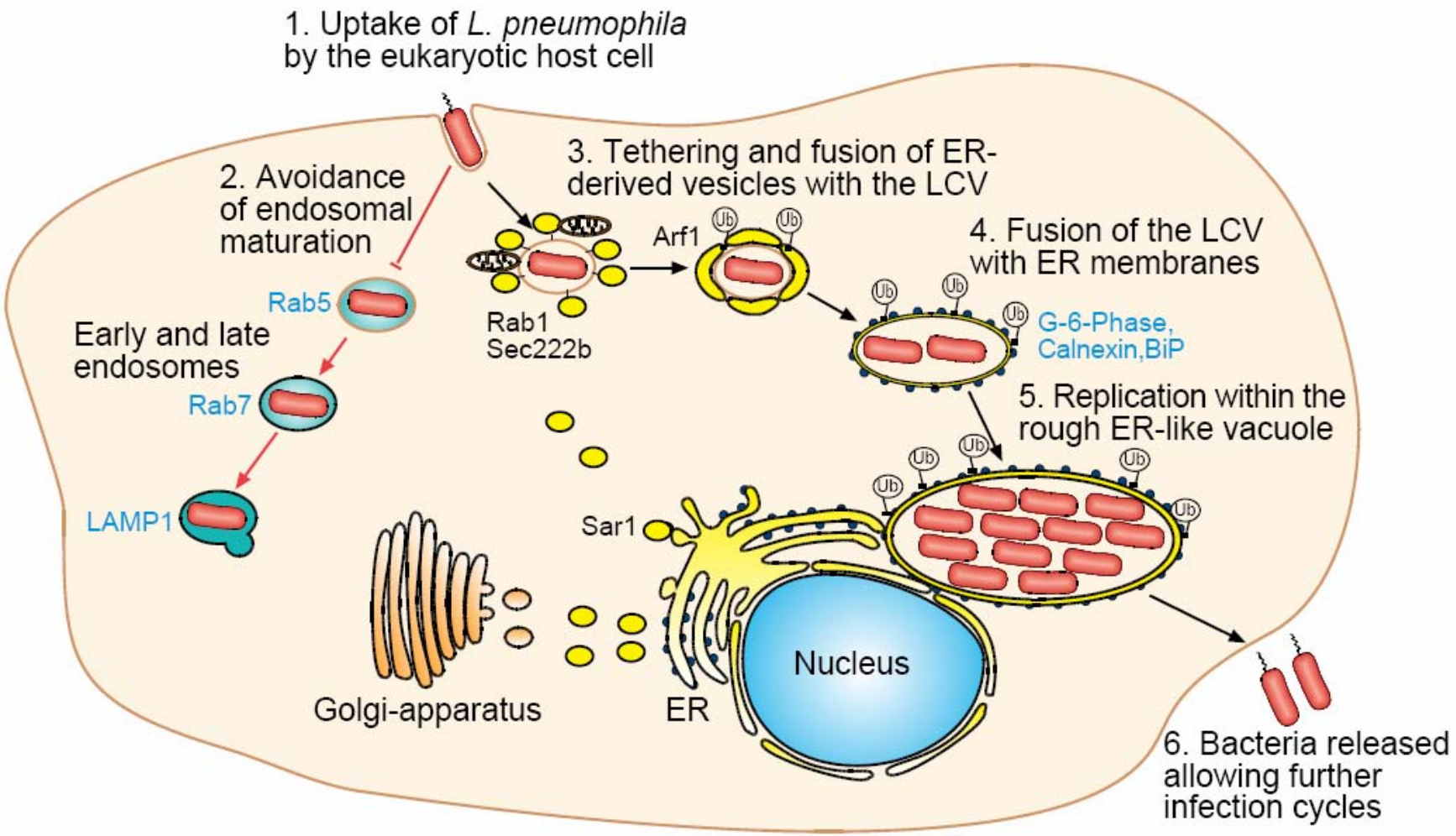
Nagai, H. & Roy, C. R.
A bacterial guanine nucleotide exchange factor activates ARF on *Legionella* phagosomes. Science 295, 679–682 (2002).
First study to show that *L. pneumophila* targets a host cell vesicle-trafficking factor through the action of a Dot/Icm translocated substrate.

Müller MP & Itzen A
The *Legionella* effector protein DrrA AMPylates the membrane traffic regulator Rab1b. Science 329, 946-949 (2010).

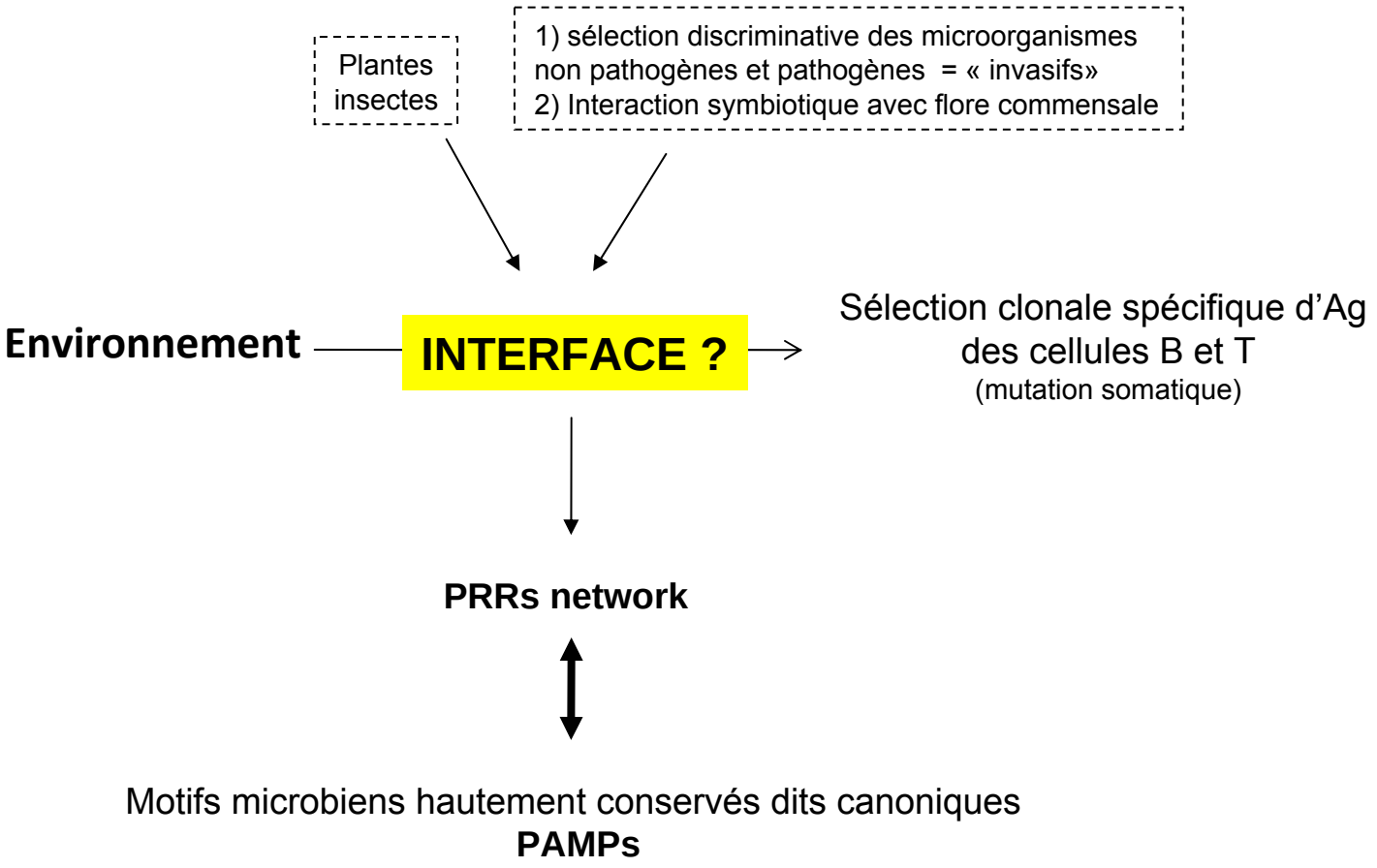


Protein	Gene locus	Domain or function	Evidence for translocation
Substrates based on similarity to eukaryotic proteins			
RalF[33]	*lpg1950*	Sec7 homology domain and Arf1 GEF; Arf1 recruitment	Cya-fusion assay and immunofluorescence microscopy
LepA[62]	*lpg2793*	Homology to EEA1, USO1, SNAREs and coiled-coil domain; bacterial egress	Cya-fusion assay and fusions to β-lactamase[125]
LepB[62,78]	*lpg2490*	Homology to EEA1, USO1, SNAREs, coiled-coil domain and Rab1 GAP; vesicle trafficking and bacterial egress	Cya-fusion assay
LegA8 (also known as AnkN and AnkX)[61,63,124]	*lpg0695*	Ankyrin repeat	Cya-fusion assay and fusions to β-lactamase[125]
LegAU13 (also known as Ceg27 and AnkB)[61,65,124]	*lpg2144*	F box; ankyrin repeat	Fusions to β-lactamase[125]
LegC8 (also known as Lgt2)[61]	*lpg2862*	Glucosyltransferase; coiled-coil domain	Fusions to β-lactamase[125]
LegL3 (REF. 61)	*lpg1660*	Leucine-rich repeat	Cya-fusion assay and fusions to β-lactamase[125]
LegLC8 (REF. 61)	*lpg1890*	Leucine-rich repeat; coiled-coil domain	Cya-fusion assay and fusions to β-lactamase[125]
LegG2 (REF. 61)	*lpg0276*	Ras GEF	Cya-fusion assay and fusions to β-lactamase[125]
LegP[31,61]	*lpg2999*	Astacin protease	Fusions to β-lactamase[125]
LegT[61]	*lpg1328*	Thaumatin domain	Fusions to β-lactamase[125]
LegU1 (REF. 61)	*lpg0171*	F box	Fusions to β-lactamase[125]
Substrates identified by directly assaying for Dot/Icm-dependent translocation			
SidF[39,73,81]	*lpg2584*	Bcl2-rambo and BNIP3 binding domain; anti-apoptosis	Inter-bacterial transfer, immunofluorescence microscopy and cya-fusion assay
SdhA[59]	*lpg0376*	Coiled-coil domain; anti-apoptosis	Saponin extraction
Substrates identified in yeast ectopic overexpression studies			
VipA[64]	*lpg0390*	Formin homology domain; vesicle trafficking	Cya-fusion assay
YlfA (also known as LegC7)[57,61]	*lpg2298*	Coiled-coil domain; vesicle trafficking	Cya-fusion assay and fusions to β-lactamase[125]
Substrates identified based on regulatory networks			
Ceg10 (REF. 65)	*lpg0284*	Hypothetical protein	Cya-fusion assay
Substrate identified by a putative Dot/Icm translocation signal			
Putative uncharacterized protein[67]	*lpg0045*	Hypothetical protein	Cya-fusion assay
Substrates identified by other mechanisms			
SidM (also known as DrrA)[76,77]	*lpg2464*	Rab1 GEF and Rab1 GDI; Rab1 recruitment	Cya-fusion assay and immunofluorescence microscopy
LidA[35,76]	*lpg0940*	Coiled-coil domain; Rab1 sequestration	Immunofluorescence microscopy and PNS
SidJ[80]	*lpg2155*	Endoplasmic reticulum recruitment	Saponin extraction and SidC-based translocation assay
WipA[39]	lpg2718	Hypothetical protein	Cya-fusion assay

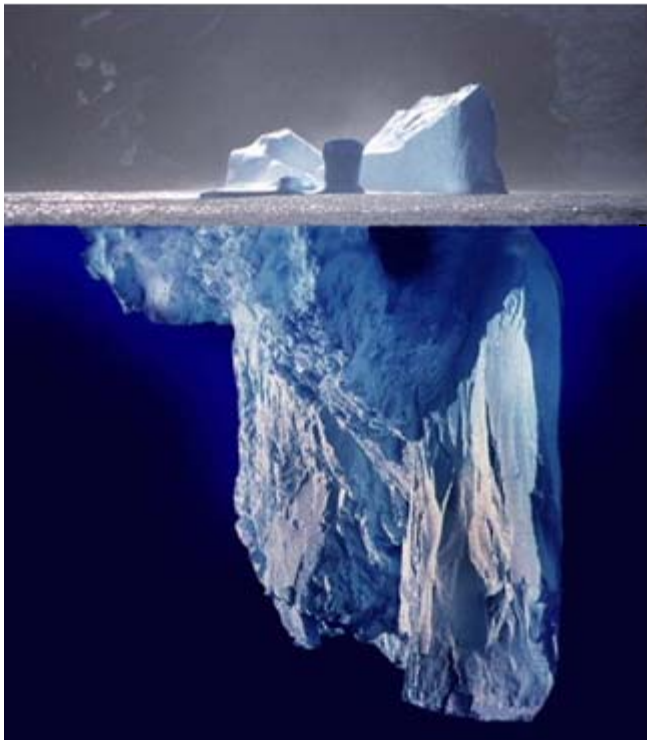
*A complete list of the Dot/Icm translocated substrates is provided in Supplementary information S1 (table). Arf1, ADP-ribosylation factor 1; Bcl2, B-cell lymphoma 2; EEA1, early endosomal antigen 1; GAP, GTPase activating protein; GDI, guanine nucleotide dissociation inhibitor; GEF, guanine nucleotide exchange factor; PNS, protein present on phagosomes isolated from post-nuclear supernatants of infected cells; RalF, recruitment of Arf1 to *Legionella* phagosome; SdhA, *sidH* paralogue A; Sid, substrates of Icm/Dot.

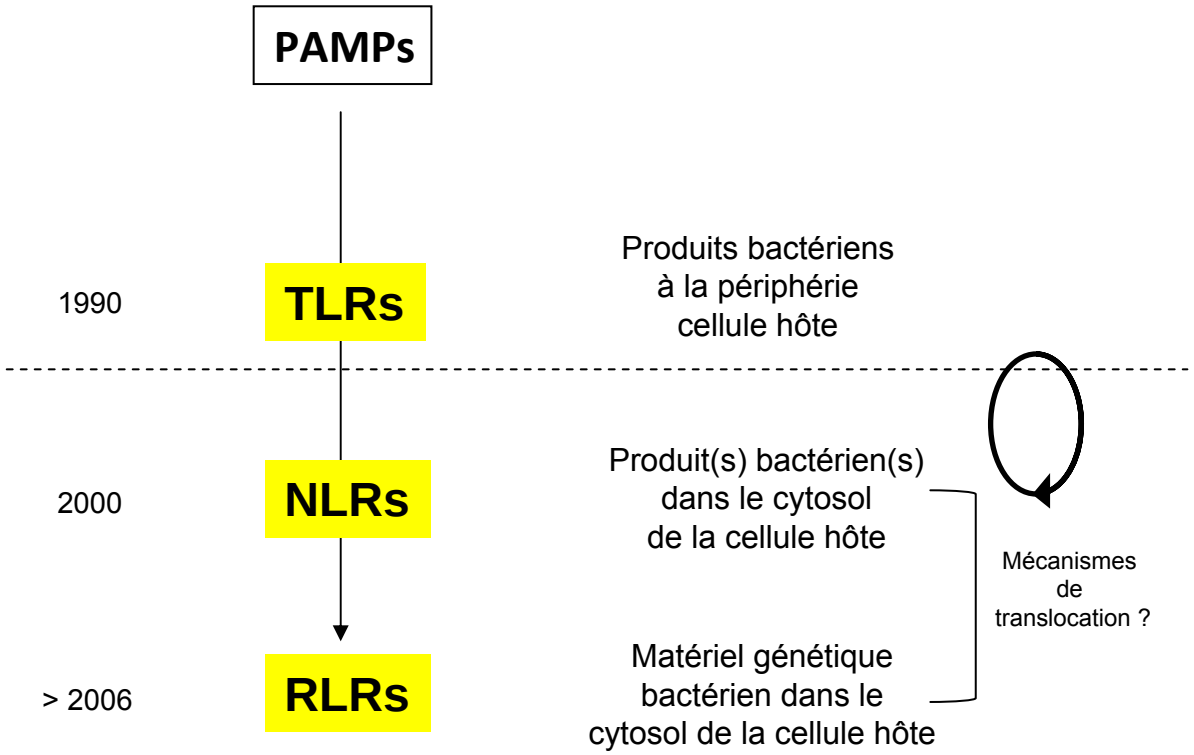

1. Uptake of *L. pneumophila* by the eukaryotic host cell
2. Avoidance of endosomal maturation
Rab5
Early and late endosomes
Rab7
LAMP1
3. Tethering and fusion of ER-derived vesicles with the LCV
Rab1
Sec222b
Arf1
Ub
4. Fusion of the LCV with ER membranes
G-6-Phase, Calnexin,BiP
5. Replication within the rough ER-like vacuole
Sar1
Golgi-apparatus
ER
Nucleus
6. Bacteria released allowing further infection cycles

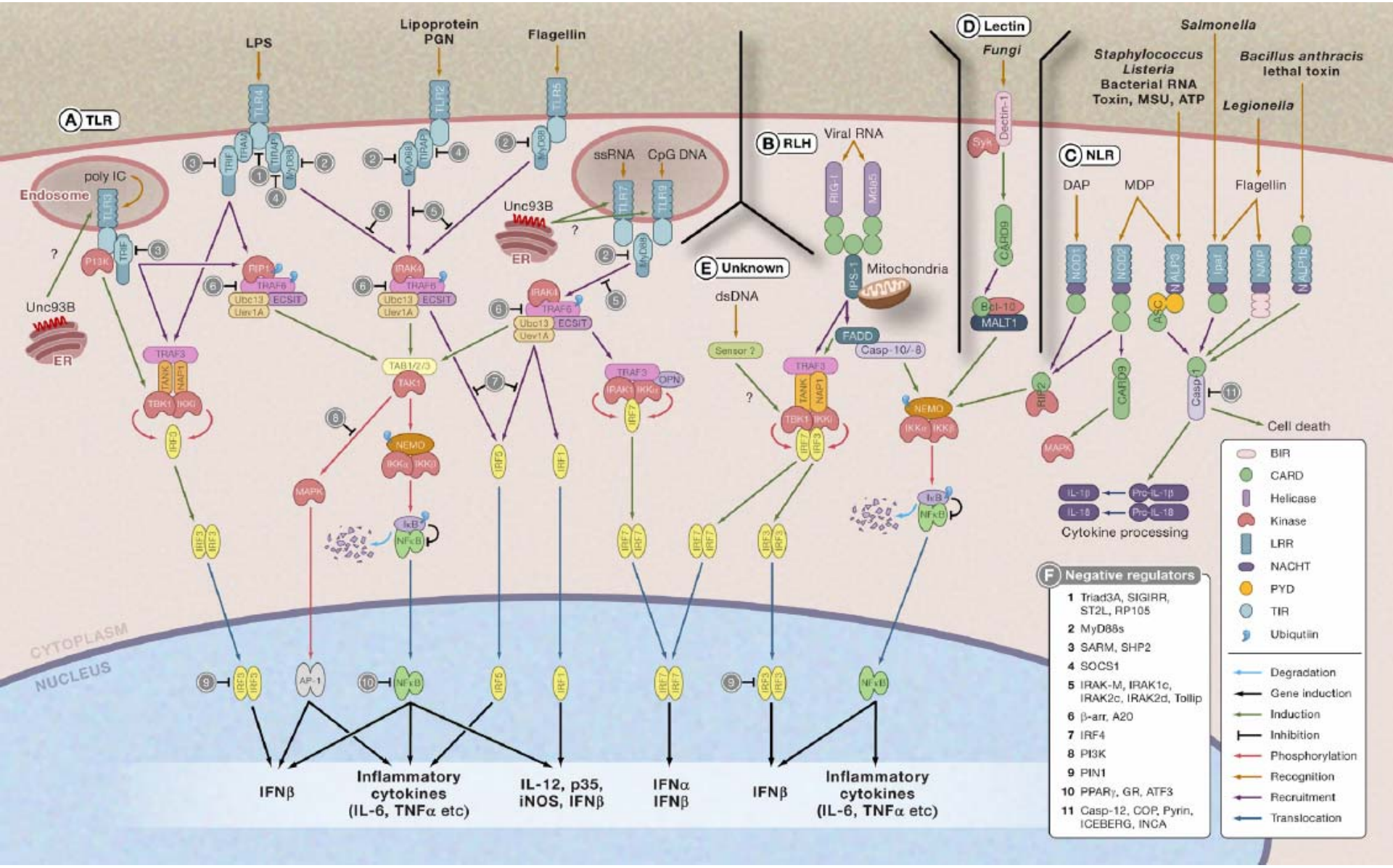
3ème partie: *Legionella* et immunité innée mammifère humaine



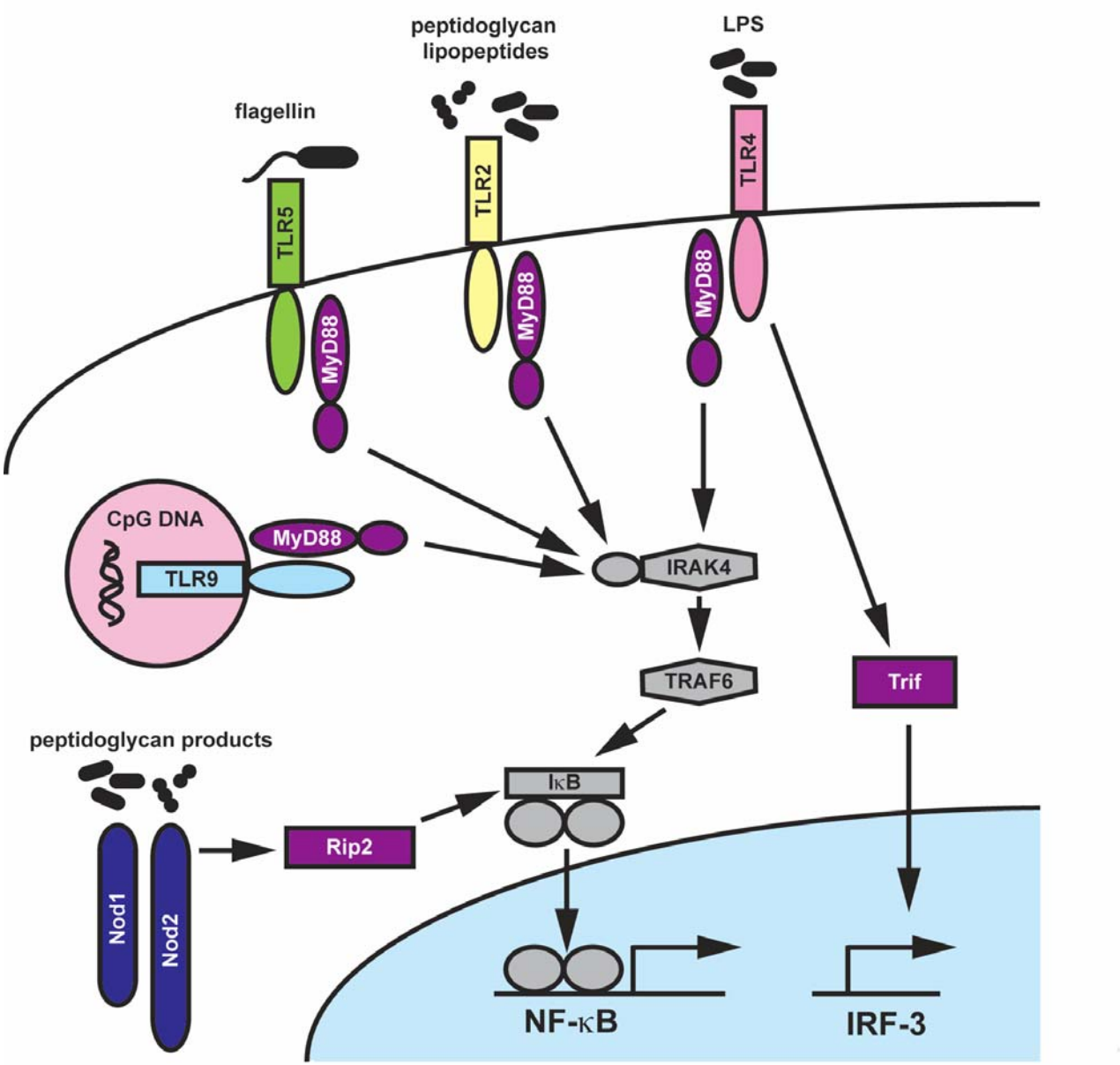
Macrophages



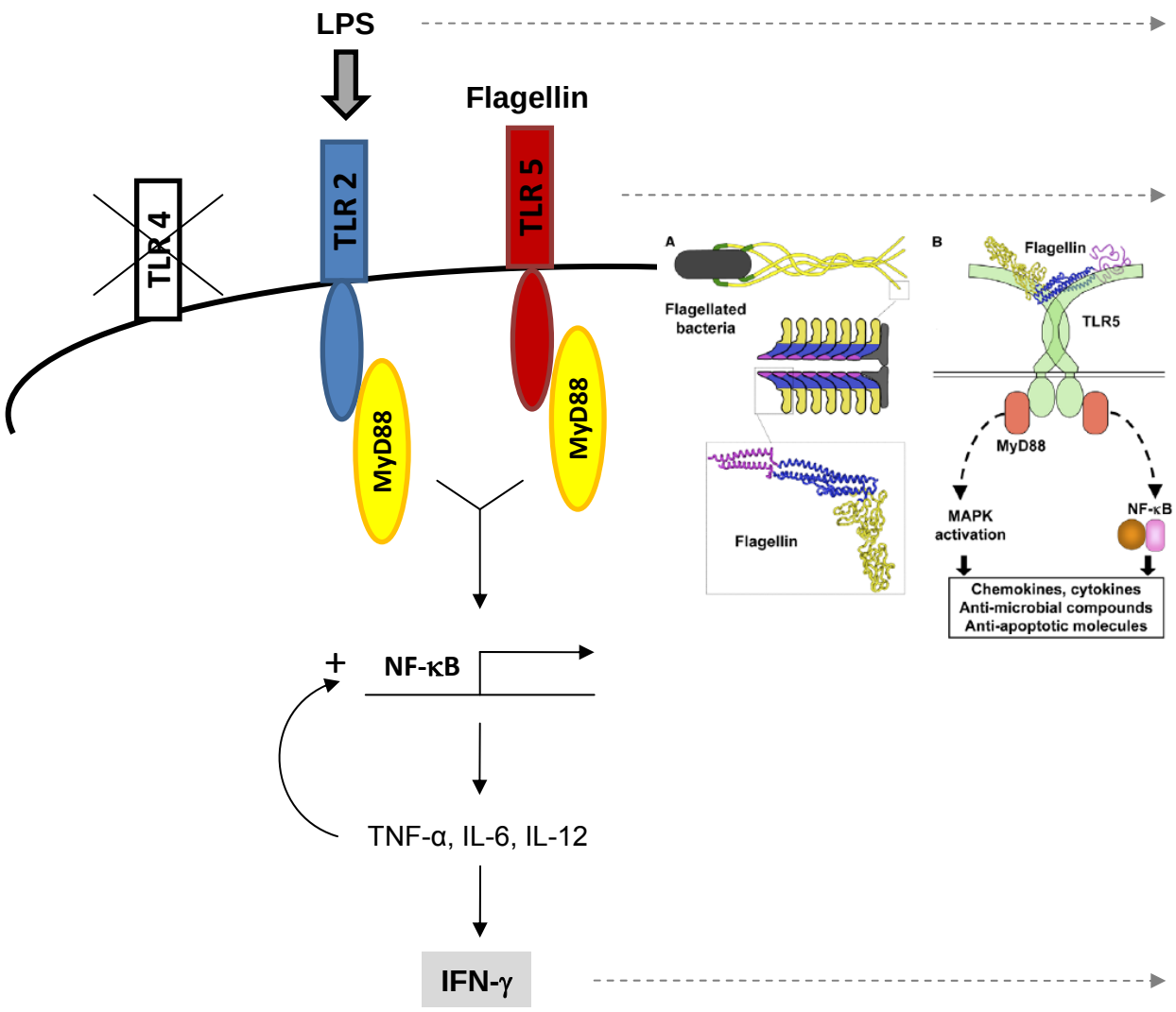



A TLR
LPS
Lipoprotein
PGN
Flagellin
TLR4
TLR2
TLR5
poly IC
Endosome
TLR3
TRIF
TRAM
TIRAP
MyD88
P13K
RIP1
TRAF6
Ubc13
Uev1A
ECSIT
IRAK4
IRAK1
TRAF3
TANK
NAP1
TBK1
IKKi
IRF3
TAB1/2/3
TAK1
NEMO
IKKα
IKKβ
IκB
NFκB
MAPK
AP-1
IRF5
IRF1
IRF7
OPN
Unc93B
ER
ssRNA
CpG DNA
TLR7
TLR9
B RLH
Viral RNA
RIG-I
MDA5
IPS-1
Mitochondria
E Unknown
dsDNA
Sensor ?
D Lectin
Fungi
Syk
Dectin-1
CARD9
Bcl-10
MALT1
Salmonella
Staphylococcus
Listeria
Bacterial RNA
Toxin, MSU, ATP
Bacillus anthracis
lethal toxin
Legionella
C NLR
DAP
MDP
Flagellin
NOD1
NOD2
NALP3
Ipaf
NAIP
NALP1b
ASC
RIP2
Casp-1
Cell death
IL-1β
Pro-IL-1β
IL-18
Pro-IL-18
Cytokine processing
FADD
Casp-10/-8
CYTOPLASM
NUCLEUS
IFNβ
Inflammatory
cytokines
(IL-6, TNFα etc)
IL-12, p35,
iNOS, IFNβ
IFNα
IFNβ
Inflammatory
cytokines
(IL-6, TNFα etc)
F Negative regulators
1 Triad3A, SIGIRR, ST2L, RP105
2 MyD88s
3 SARM, SHP2
4 SOCS1
5 IRAK-M, IRAK1c, IRAK2c, IRAK2d, Tollip
6 β-arr, A20
7 IRF4
8 PI3K
9 PIN1
10 PPARγ, GR, ATF3
11 Casp-12, COP, Pyrin, ICEBERG, INCA
BIR
CARD
Helicase
Kinase
LRR
NACHT
PYD
TIR
Ubiquitin
Degradation
Gene induction
Induction
Inhibition
Phosphorylation
Recognition
Recruitment
Translocation

Pattern recognition receptors (PRRs) detect conserved molecular patterns (PAMPs) on microbes



TLRs



Lipid A structure

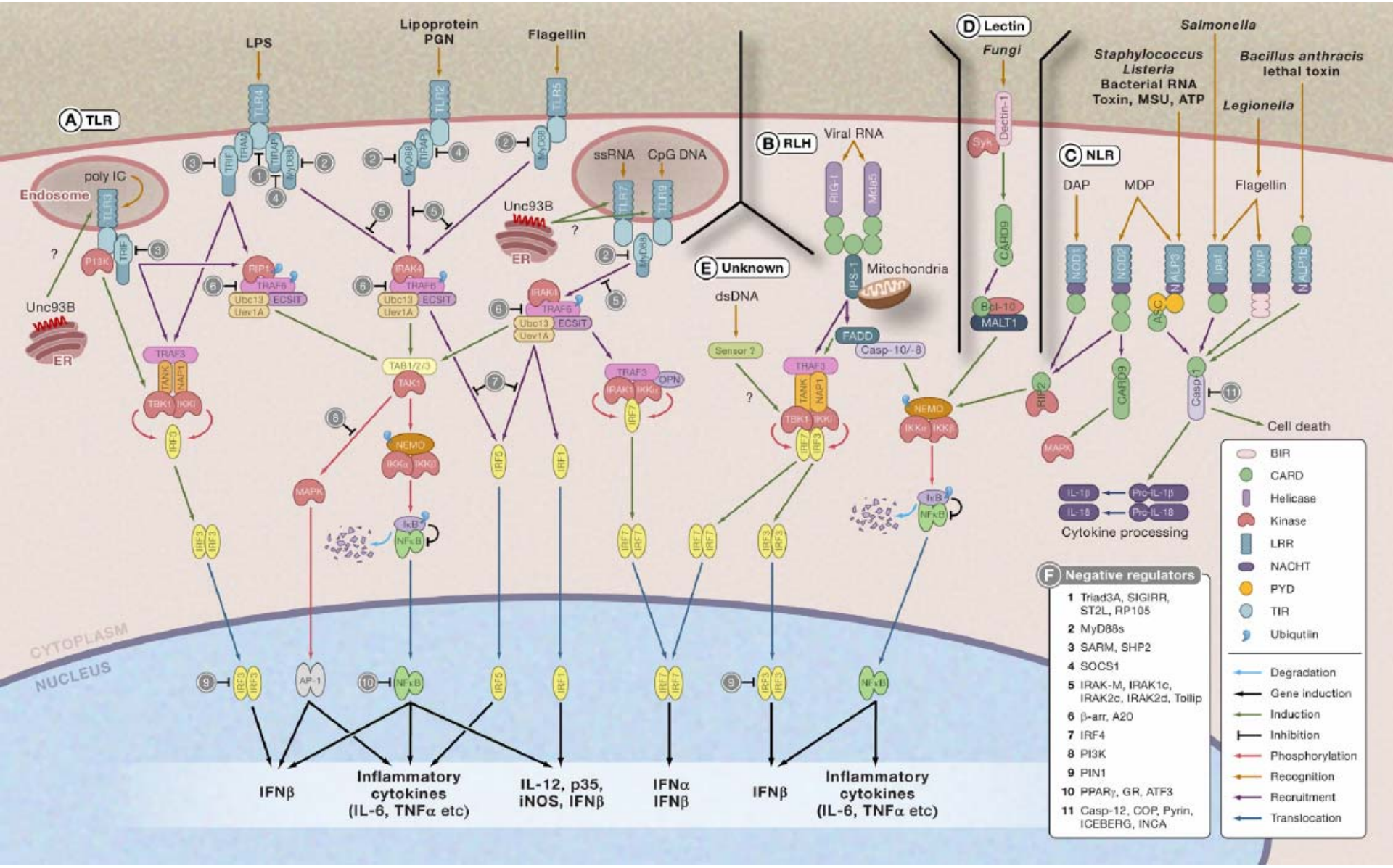
Lettinga KD, 2002a ; Girard R, 2003

TLR5 Polymorphism

Lettinga KD, 2002b ; Hawn TR, 2003

IFN-γ Hyporesponsiveness

Lettinga KD, 2003


A TLR
LPS
Lipoprotein
PGN
Flagellin
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Endosome
TLR3
TRIF
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TIRAP
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P13K
RIP1
TRAF6
Ubc13
Uev1A
ECSIT
IRAK4
IRAK1
TRAF3
TANK
NAP1
TBK1
IKKi
IRF3
TAB1/2/3
TAK1
NEMO
IKKα
IKKβ
IκB
NFκB
MAPK
AP-1
IRF5
IRF1
IRF7
OPN
Unc93B
ER
ssRNA
CpG DNA
TLR7
TLR9
B RLH
Viral RNA
RIG-I
MDA5
IPS-1
Mitochondria
E Unknown
dsDNA
Sensor ?
D Lectin
Fungi
Syk
Dectin-1
CARD9
Bcl-10
MALT1
Salmonella
Staphylococcus
Listeria
Bacterial RNA
Toxin, MSU, ATP
Bacillus anthracis
lethal toxin
Legionella
C NLR
DAP
MDP
Flagellin
NOD1
NOD2
NALP3
Ipaf
NAIP
NALP1b
ASC
RIP2
Casp-1
Cell death
IL-1β
Pro-IL-1β
IL-18
Pro-IL-18
Cytokine processing
FADD
Casp-10/-8
CYTOPLASM
NUCLEUS
IFNβ
Inflammatory
cytokines
(IL-6, TNFα etc)
IL-12, p35,
iNOS, IFNβ
IFNα
IFNβ
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cytokines
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F Negative regulators
1 Triad3A, SIGIRR, ST2L, RP105
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5 IRAK-M, IRAK1c, IRAK2c, IRAK2d, Tollip
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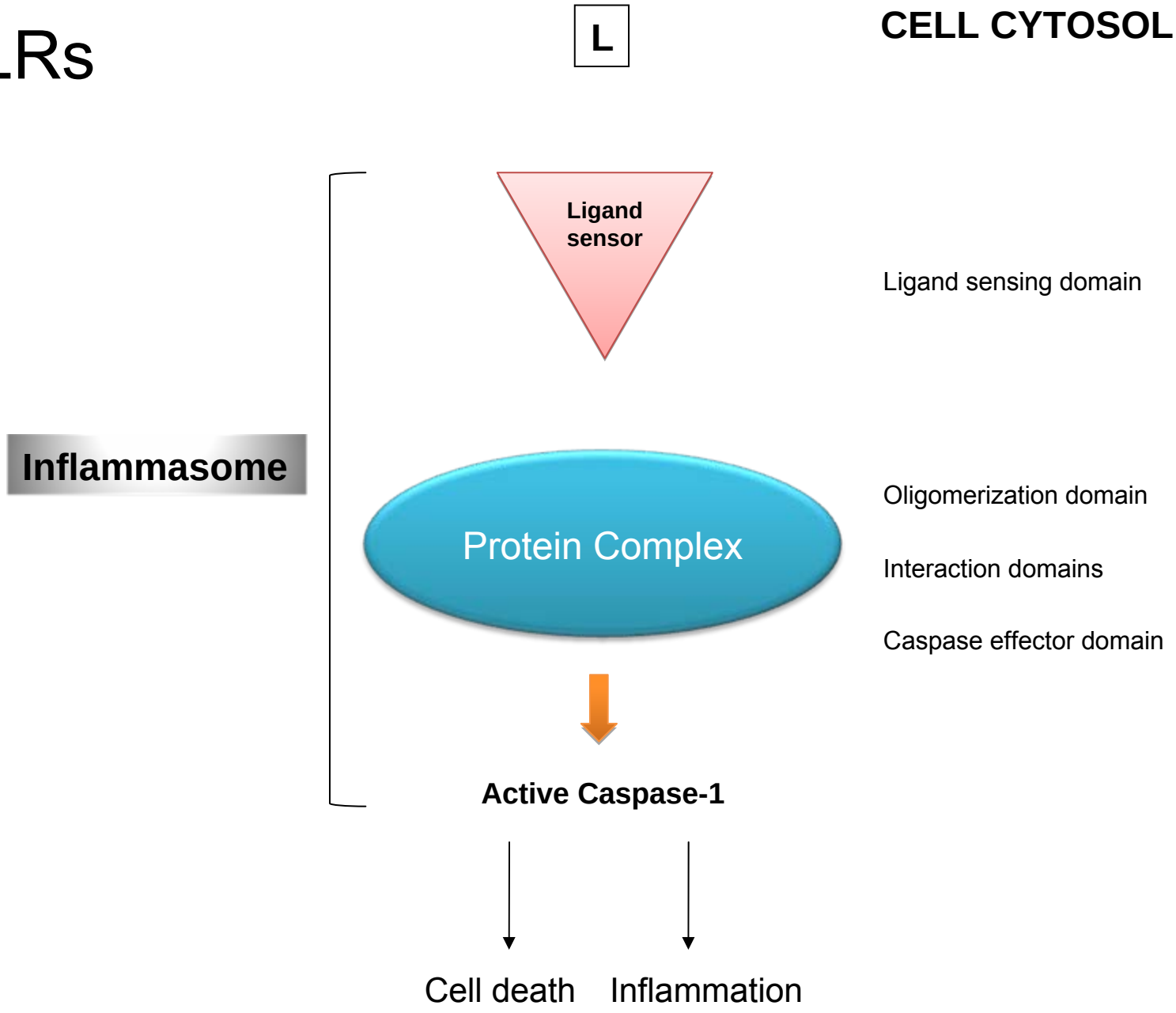
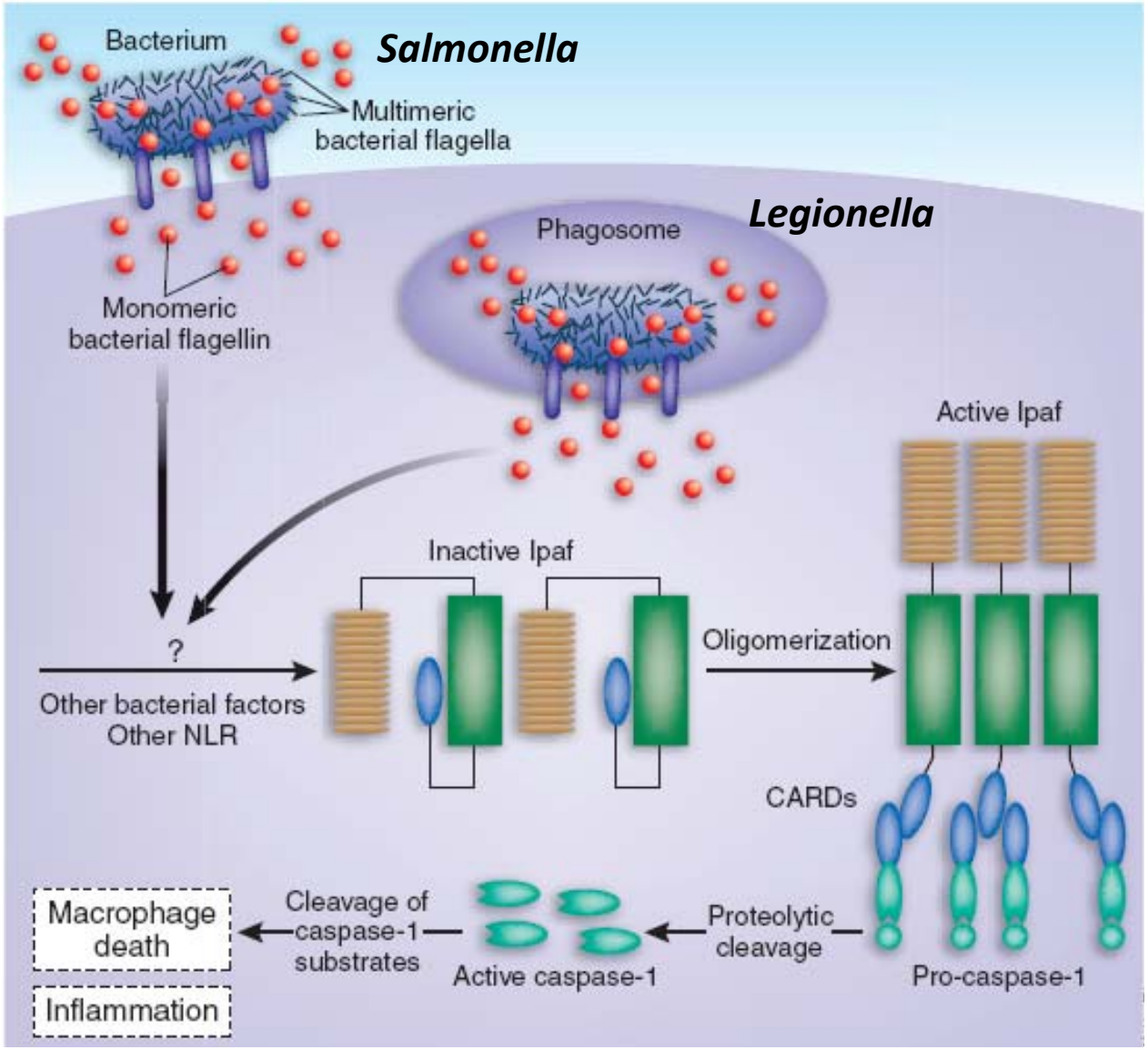
CARD
Helicase
Kinase
LRR
NACHT
PYD
TIR
Ubiquitin
Degradation
Gene induction
Induction
Inhibition
Phosphorylation
Recognition
Recruitment
Translocation

NLRs


L
CELL CYTOSOL
Ligand sensor
Ligand sensing domain
Inflammasome
Protein Complex
Oligomerization domain
Interaction domains
Caspase effector domain
Active Caspase-1
Cell death
Inflammation



Ligand sensing domain

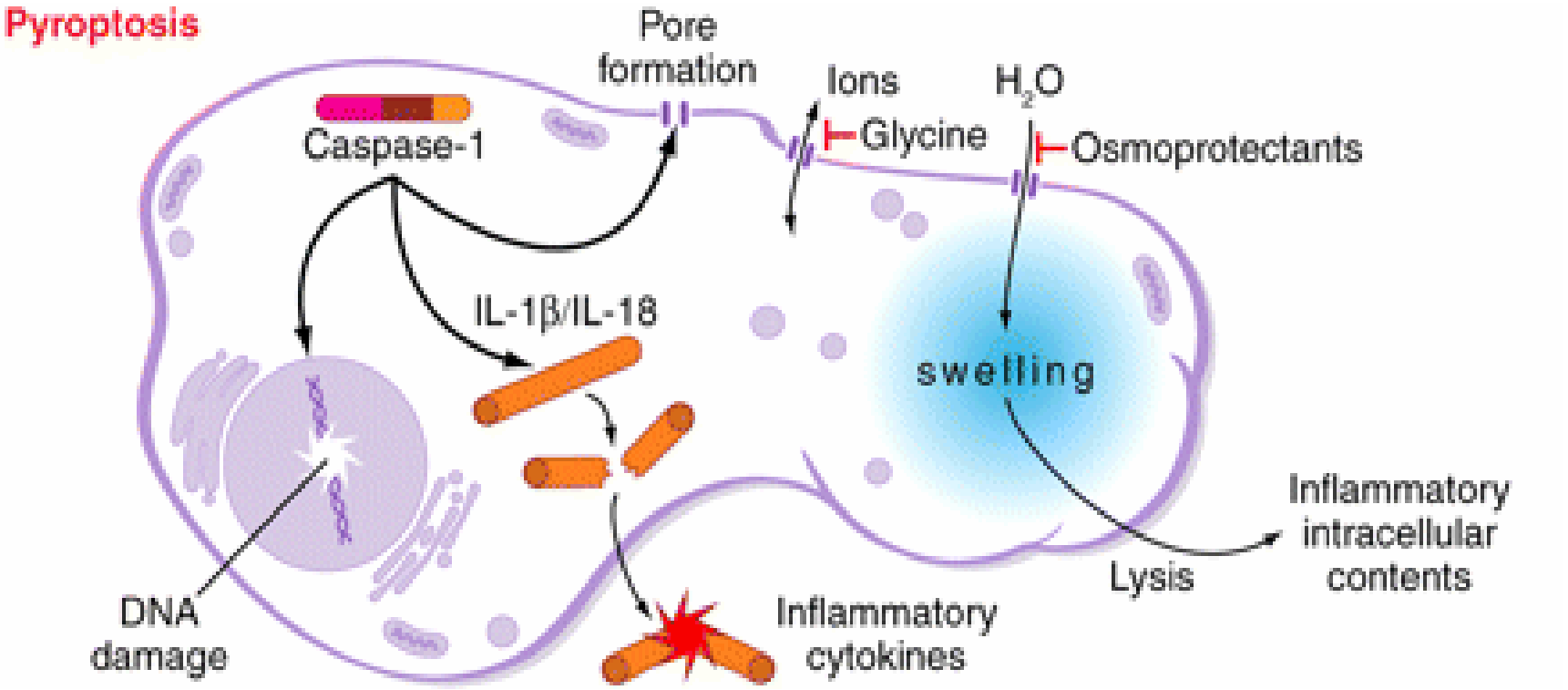
Oligomerization domain

Interaction domains

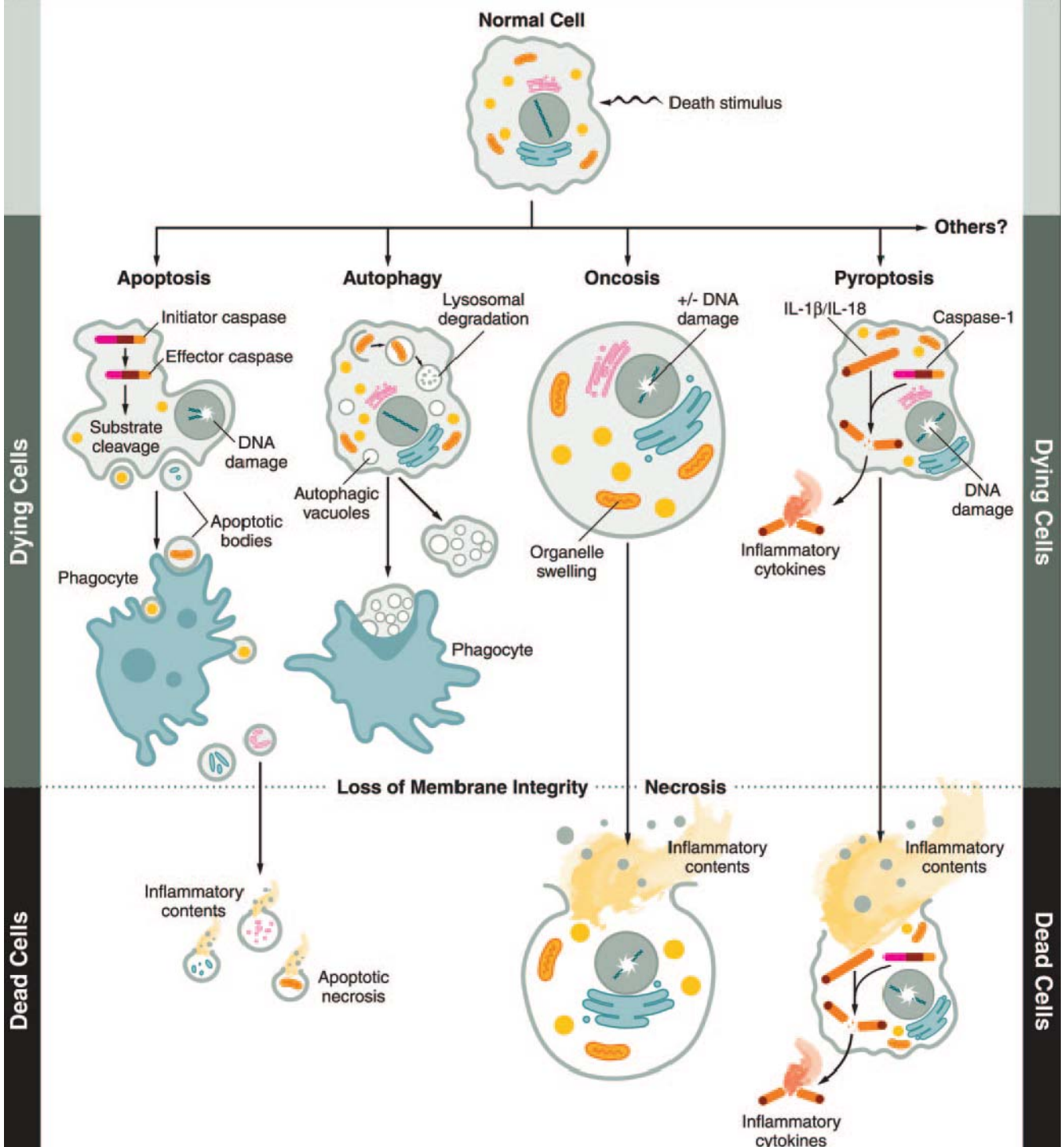
Caspase effector domain

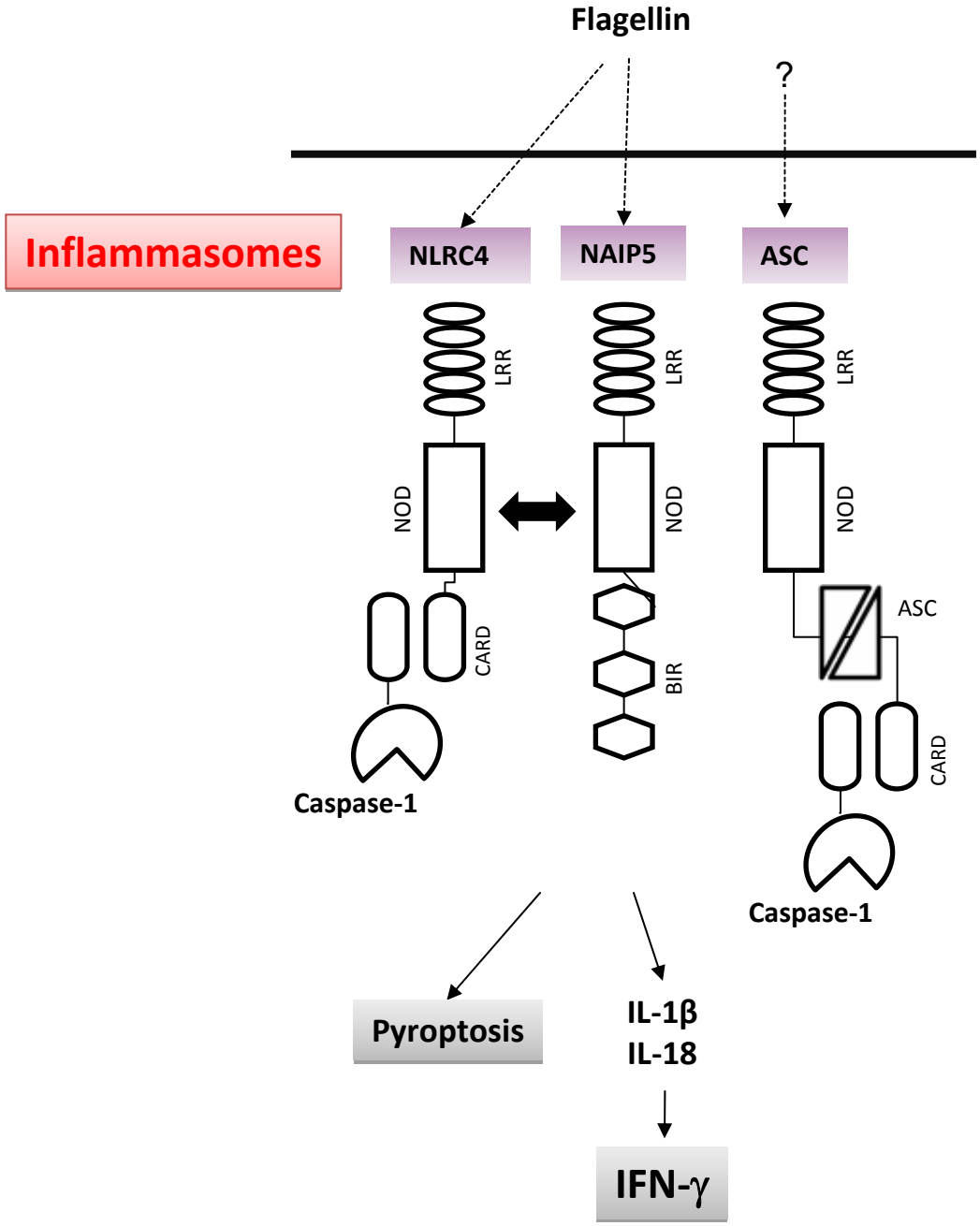
Roy CR et al., Nat Immunol 2006

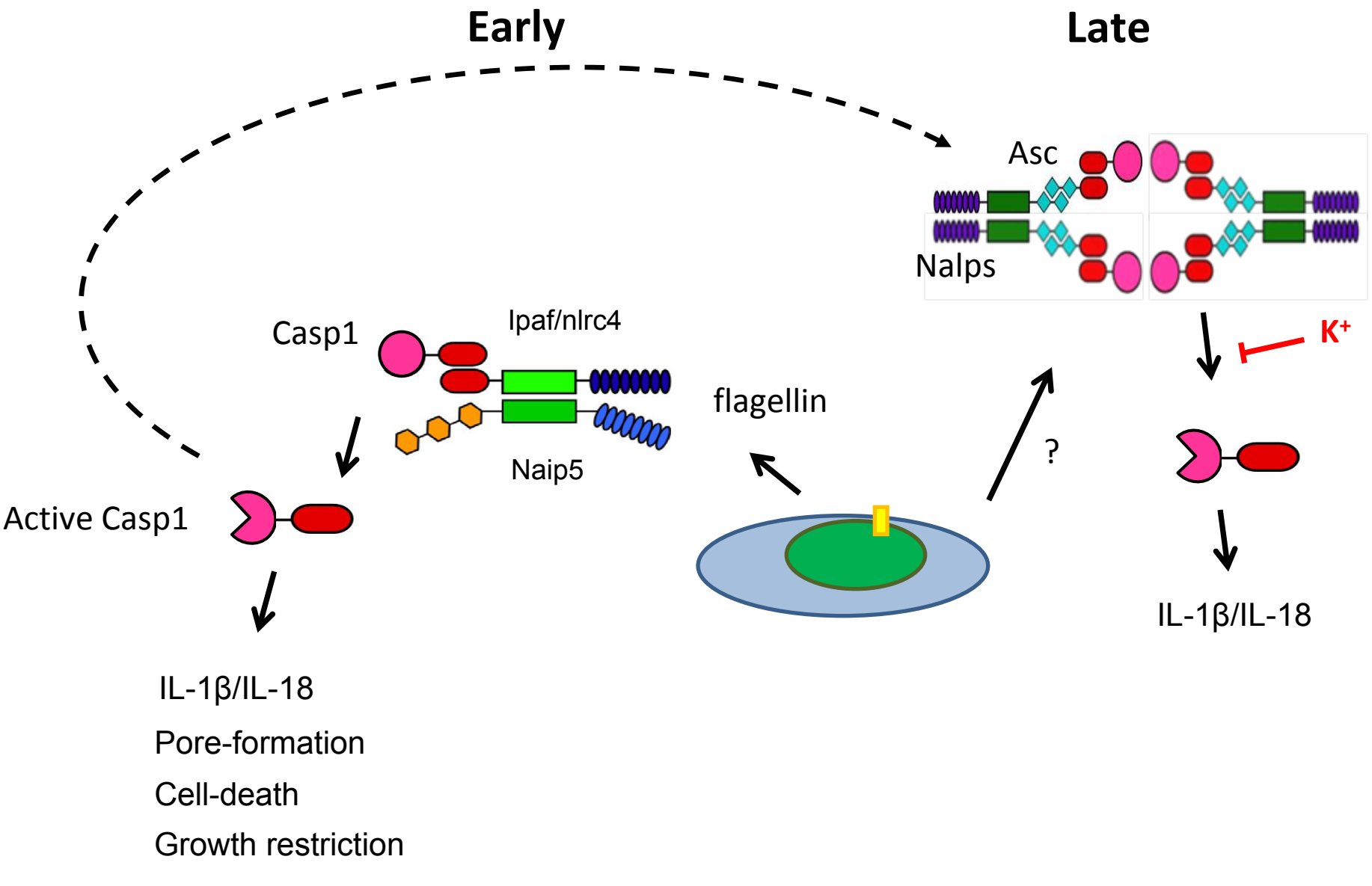
Caspase-1 functions

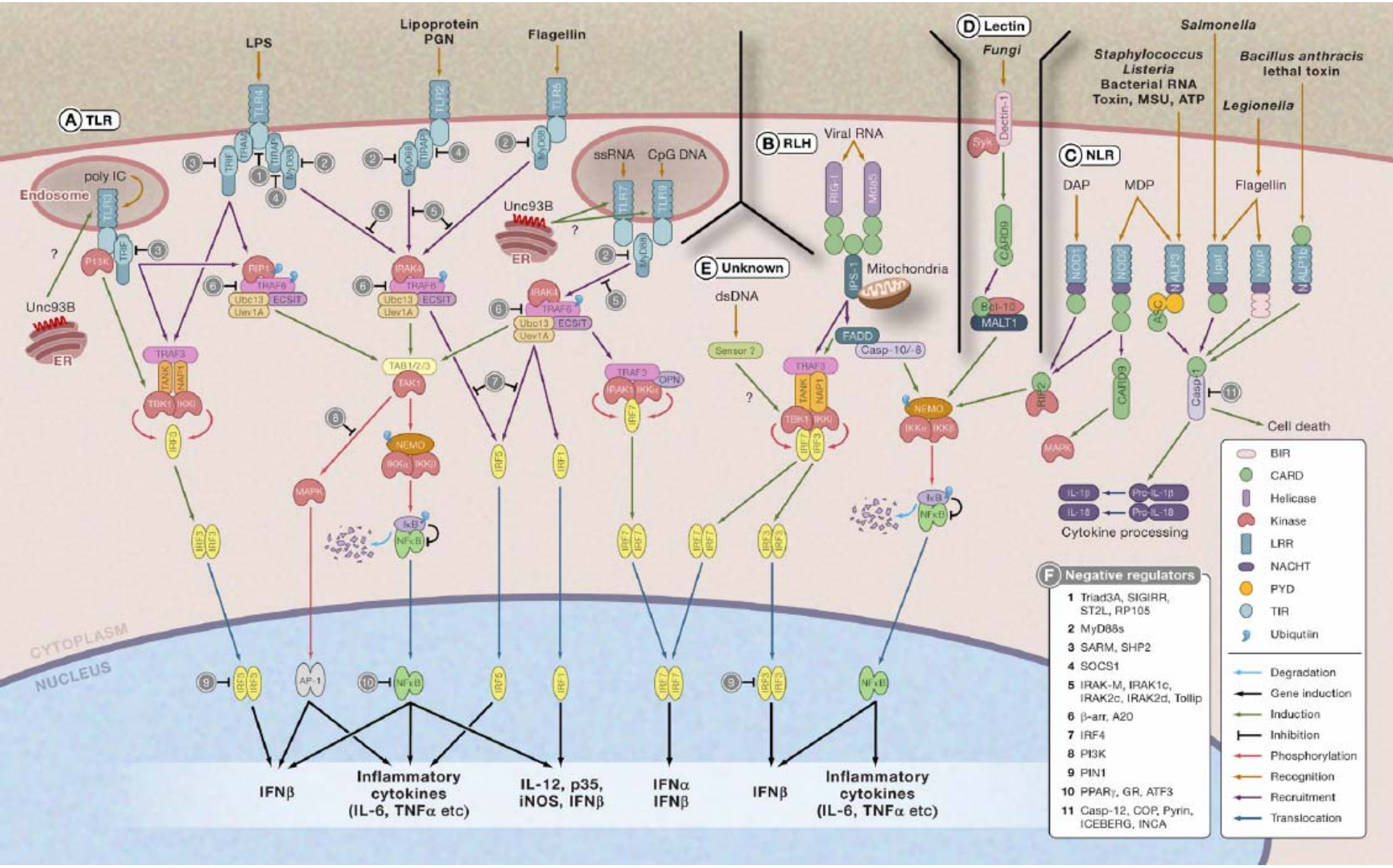


Fink, S et al. PNAS. 2008


Normal Cell
Death stimulus
Others?
Apoptosis
Autophagy
Oncosis
Pyroptosis
Initiator caspase
Effector caspase
Substrate cleavage
DNA damage
Apoptotic bodies
Phagocyte
Lysosomal degradation
Autophagic vacuoles
Phagocyte
+/- DNA damage
Organelle swelling
IL-1β/IL-18
Caspase-1
DNA damage
Inflammatory cytokines
Dying Cells
Loss of Membrane Integrity
Necrosis
Inflammatory contents
Apoptotic necrosis
Inflammatory contents
Inflammatory contents
Inflammatory cytokines
Dead Cells

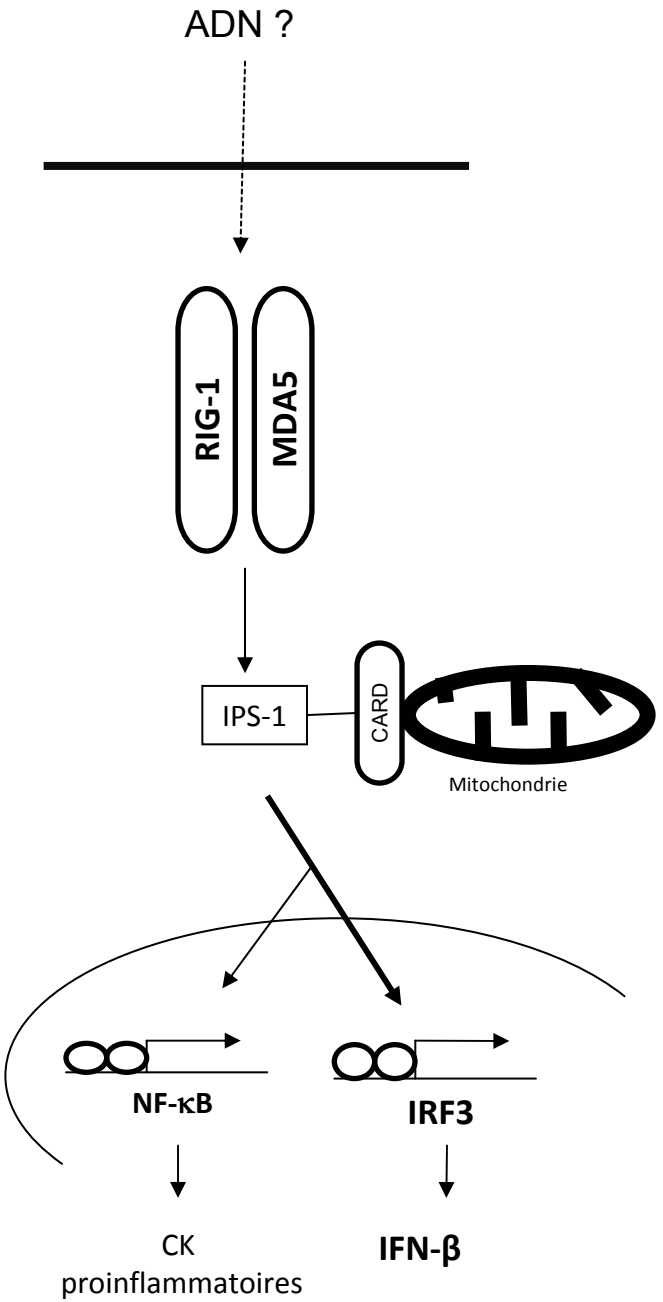

Flagellin
?
Inflammasomes
NLRC4
NAIP5
ASC
LRR
NOD
CARD
BIR
ASC
Caspase-1
Caspase-1
Pyroptosis
IL-1β
IL-18
IFN-γ


Early
Late
Asc
Nalps
Casp1
Ipaf/nlrc4
Naip5
flagellin
?
K+
Active Casp1
IL-1β/IL-18
IL-1β/IL-18
Pore-formation
Cell-death
Growth restriction


A TLR
LPS
Lipoprotein
PGN
Flagellin
TLR4
TLR2
TLR5
poly IC
Endosome
TLR3
TRIF
TRAM
TIRAP
MyD88
P13K
RIP1
TRAF6
Ubc13
Uev1A
ECSIT
IRAK4
IRAK1
TRAF3
TANK
NAP1
TBK1
IKKi
IRF3
TAB1/2/3
TAK1
NEMO
IKKα
IKKβ
IκB
NFκB
MAPK
AP-1
IRF5
IRF1
IRF7
OPN
Unc93B
ER
ssRNA
CpG DNA
TLR7
TLR9
B RLH
Viral RNA
RIG-I
MDA5
IPS-1
Mitochondria
E Unknown
dsDNA
Sensor ?
D Lectin
Fungi
Syk
Dectin-1
CARD9
Bcl-10
MALT1
Salmonella
Staphylococcus
Listeria
Bacterial RNA
Toxin, MSU, ATP
Bacillus anthracis
lethal toxin
Legionella
C NLR
DAP
MDP
Flagellin
NOD1
NOD2
NALP3
Ipaf
NAIP
NALP1b
ASC
RIP2
Casp-1
Cell death
IL-1β
Pro-IL-1β
IL-18
Pro-IL-18
Cytokine processing
FADD
Casp-10/-8
CYTOPLASM
NUCLEUS
IFNβ
Inflammatory
cytokines
(IL-6, TNFα etc)
IL-12, p35,
iNOS, IFNβ
IFNα
IFNβ
Inflammatory
cytokines
(IL-6, TNFα etc)
F Negative regulators
1 Triad3A, SIGIRR, ST2L, RP105
2 MyD88s
3 SARM, SHP2
4 SOCS1
5 IRAK-M, IRAK1c, IRAK2c, IRAK2d, Tollip
6 β-arr, A20
7 IRF4
8 PI3K
9 PIN1
10 PPARγ, GR, ATF3
11 Casp-12, COP, Pyrin, ICEBERG, INCA
BIR
CARD
Helicase
Kinase
LRR
NACHT
PYD
TIR
Ubiquitin
Degradation
Gene induction
Induction
Inhibition
Phosphorylation
Recognition
Recruitment
Translocation

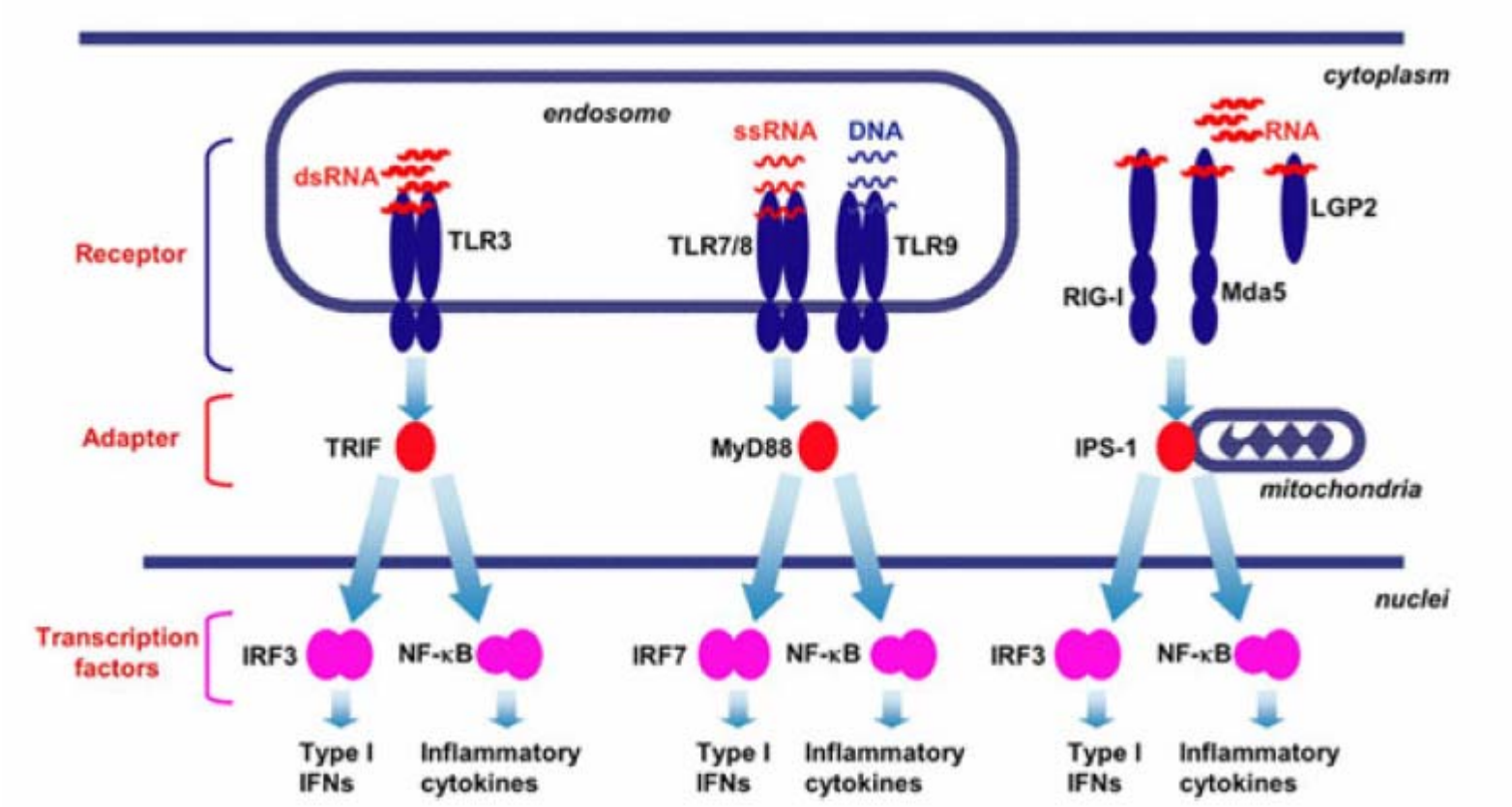
Voies de signalisation RLRs activées dans l'infection à *L. pneumophila*

Le concept d'immunité antivirale appliqué aux bactéries intracellulaires

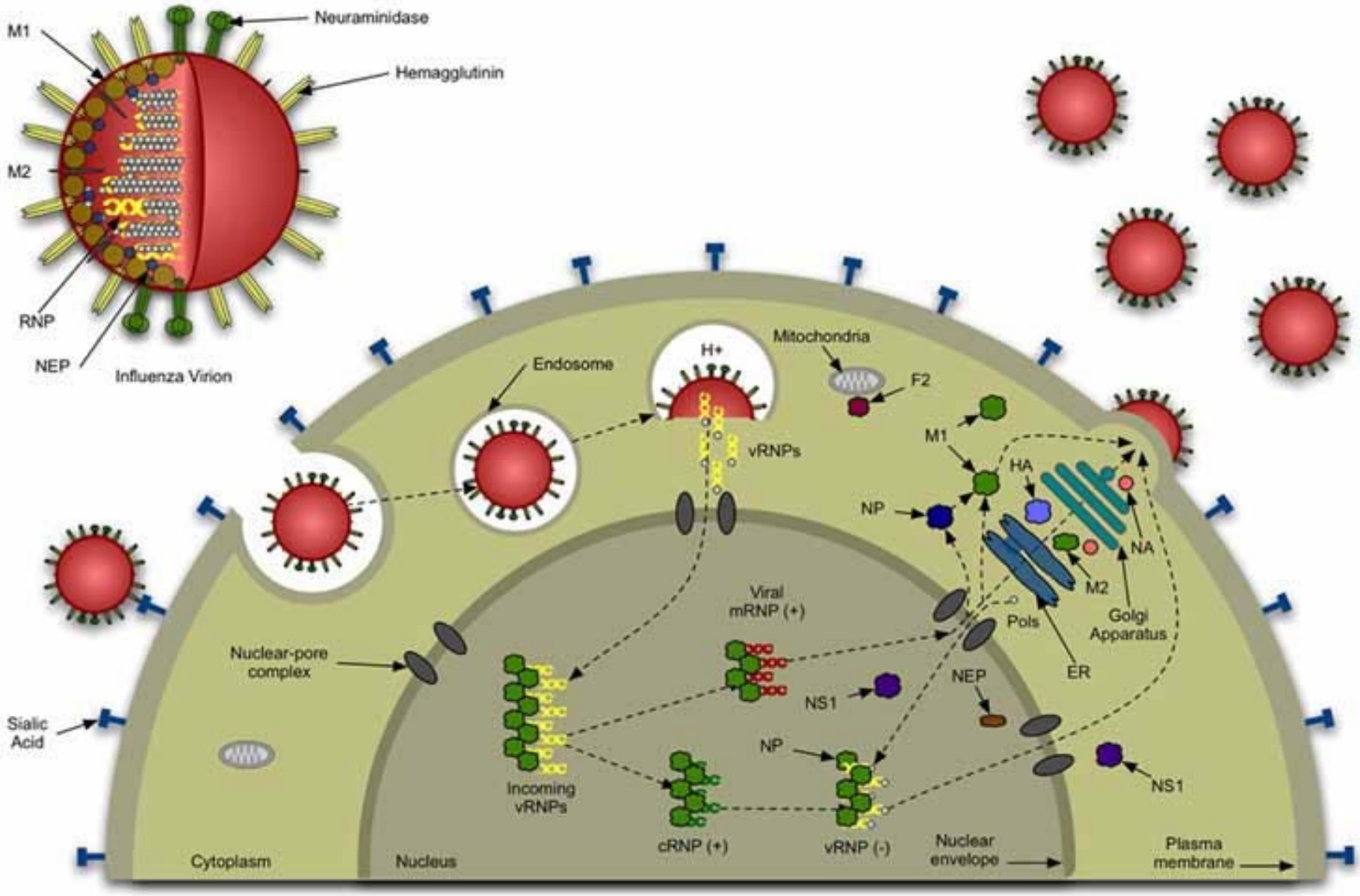


The type I IFN pathway

Recognition of viral **nucleic acids** by TLRs, RLRs and the cytosolic DNA sensor
Recognition IC bacteria: *Listeria*, *Legionella*, *Francisella*, *Mycobacteria*



IFN-stimulated genes ISG (over 400 identified by microarray analysis)
« Antiviral state » linking innate ↔ adaptive


M1
Neuraminidase
Hemagglutinin
M2
RNP
NEP
Influenza Virion
Endosome
H+
Mitochondria
F2
vRNPs
M1
HA
NP
NA
M2
Pols
Golgi Apparatus
ER
Viral mRNP (+)
Nuclear-pore complex
NS1
NEP
Sialic Acid
NP
NS1
Incoming vRNPs
cRNP (+)
vRNP (-)
Cytoplasm
Nucleus
Nuclear envelope
Plasma membrane

Au total

L'homme est un **hôte accidentel** pour *Legionella*

Le concept de **transition adaptative**:

cellule hôte environnementale → cellule hôte humaine

(immunité innée rudimentaire) (fonctions innées complexes et redondantes)

Fait émerger la contradiction entre l'aptitude bactérienne à prendre le contrôle des voies de trafficking vésiculaire et la bactéricidie exercée par la cellule hôte.

La restriction de croissance/bactéricidie est principalement médiée par la mort cellulaire de type **pyroptose**.

D'où la difficulté à générer des réponses clonales adaptatives et une immunité durable et protectrice.

Pour *Legionella*, les facteurs contribuant au « débordement » de l'immunité innée de l'hôte sont peu connus :

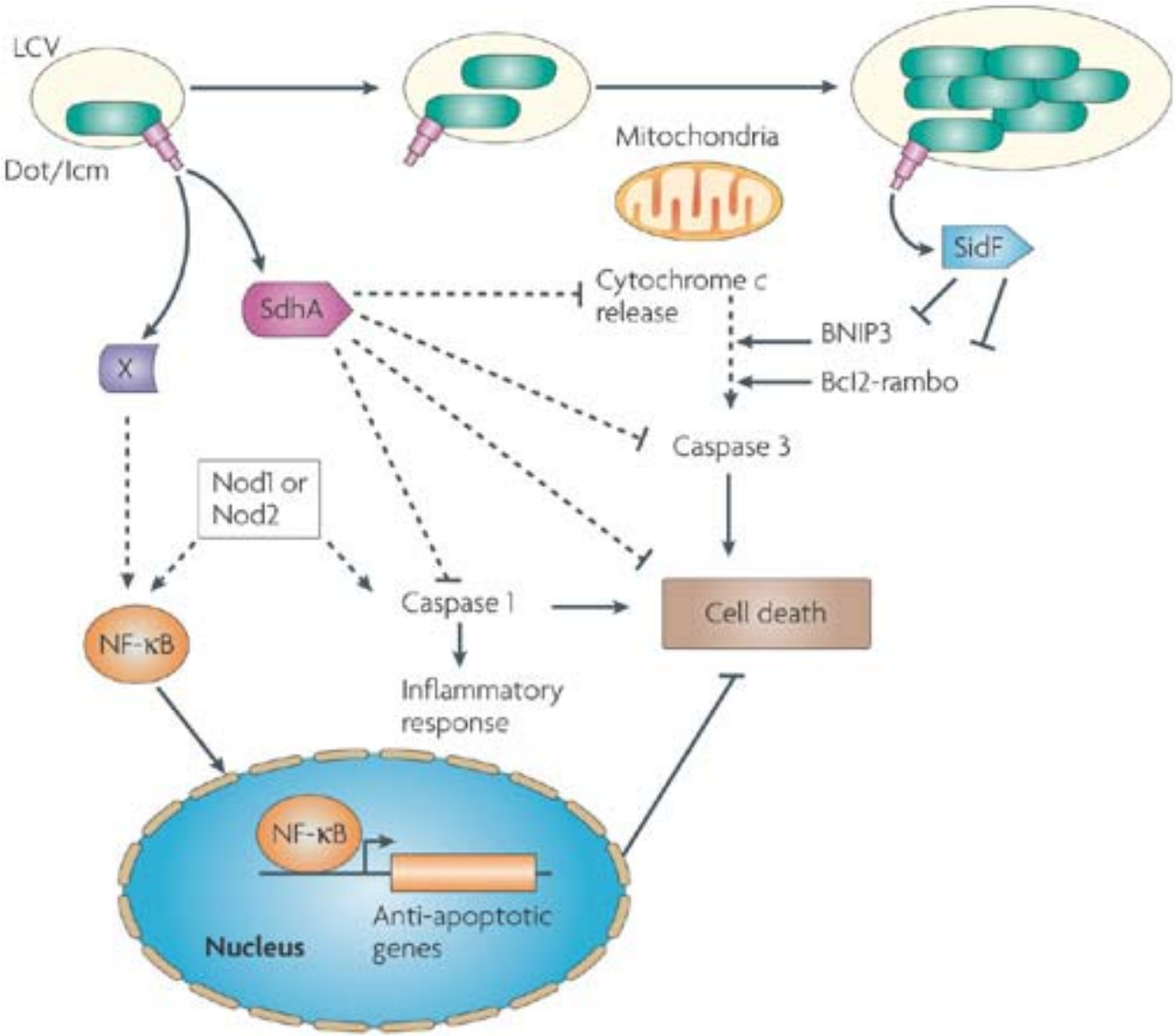
-Rôle des agents exogènes: tabac en particulier, alcool

- Déterminisme génétique (polymorphisme TLR5, IFN-γ)

-Virulence bactérienne intrinsèque :

T4SS,

vacuoles aérosolisées


LCV
Dot/Icm
Mitochondria
SidF
SdhA
Cytochrome c release
BNIP3
Bcl2-rambo
X
Caspase 3
Nod1 or Nod2
Caspase 1
Cell death
NF-κB
Inflammatory response
NF-κB
Anti-apoptotic genes
Nucleus

Le questionnaire de Proust de *Legionella*

Avec qui zonez-vous habituellement ?
Les amibes.

Quel votre point fort ?
Mon appareil de sécrétion de type IV.

Quel votre point faible ?
Mon cœur de pyrex ne résiste pas au feu de la pyroptose.

Votre boite de nuit préférée ?
Les Bains Douches.

Quelle est votre devise ?
Watch out, I'm a work in progress…