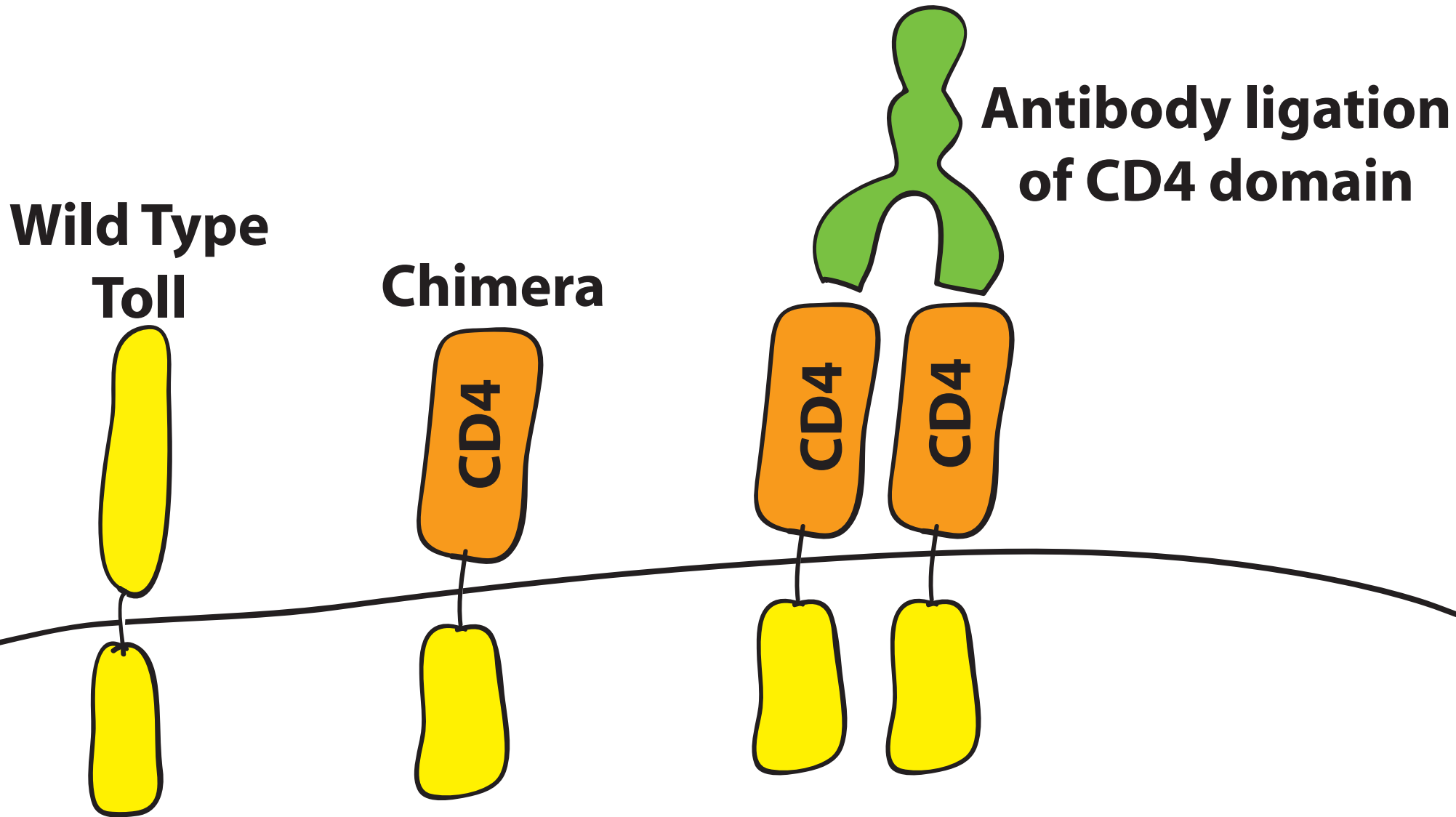


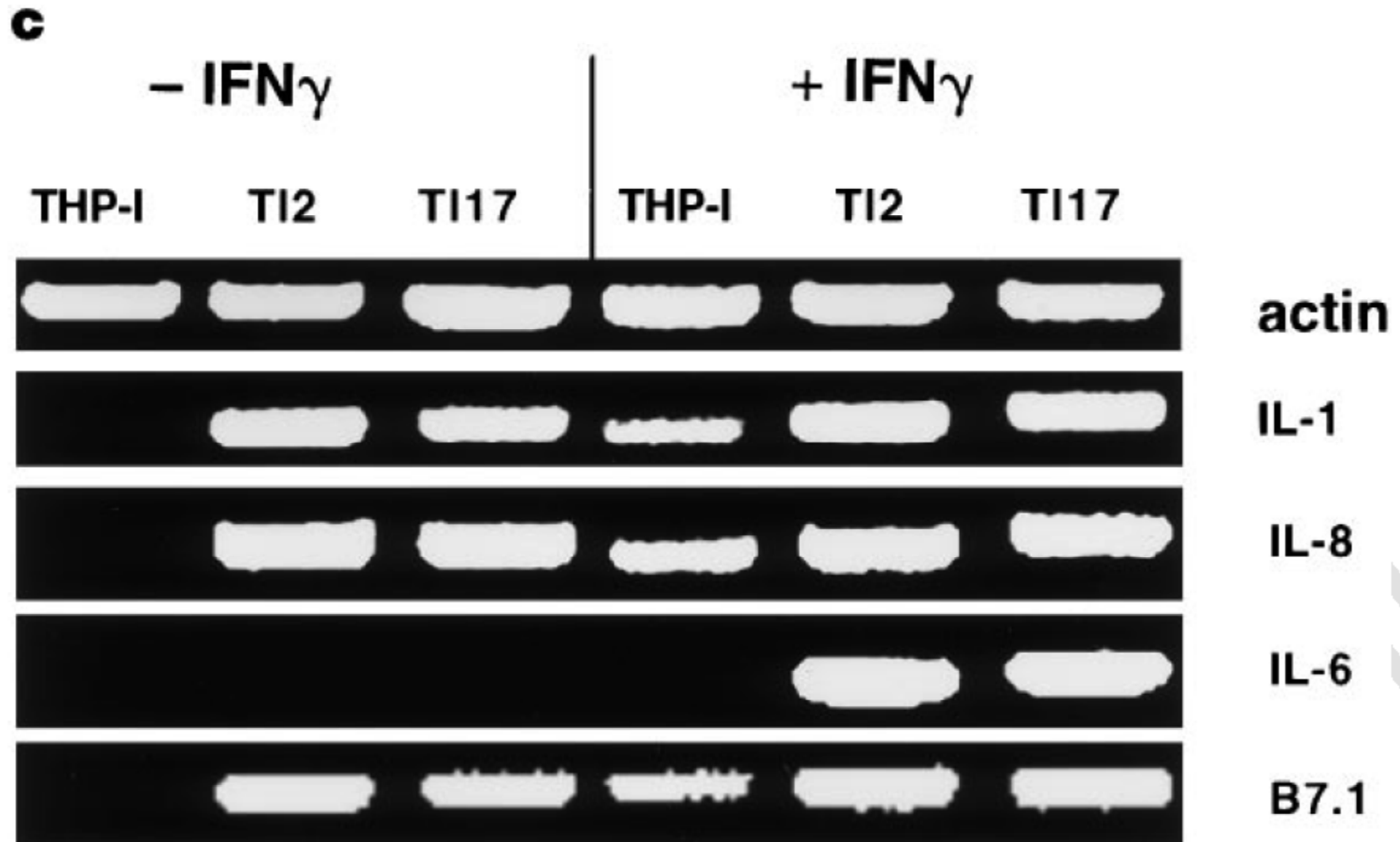
Taormina Sicily, site of Toll 2004



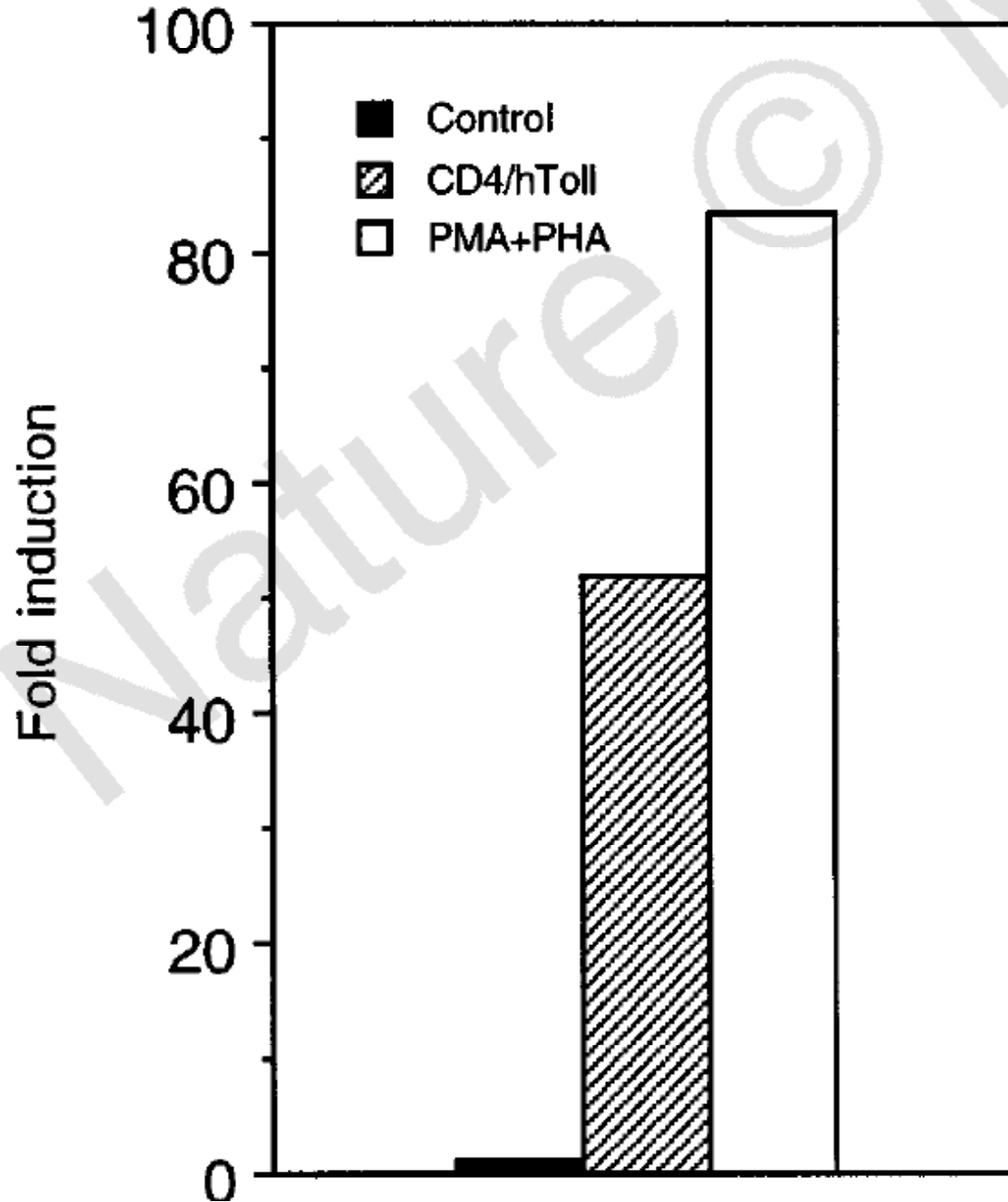
Creation of a Toll chimera to regulate activation



Toll chimeras signal constitutively



Toll chimeras activate NFkB

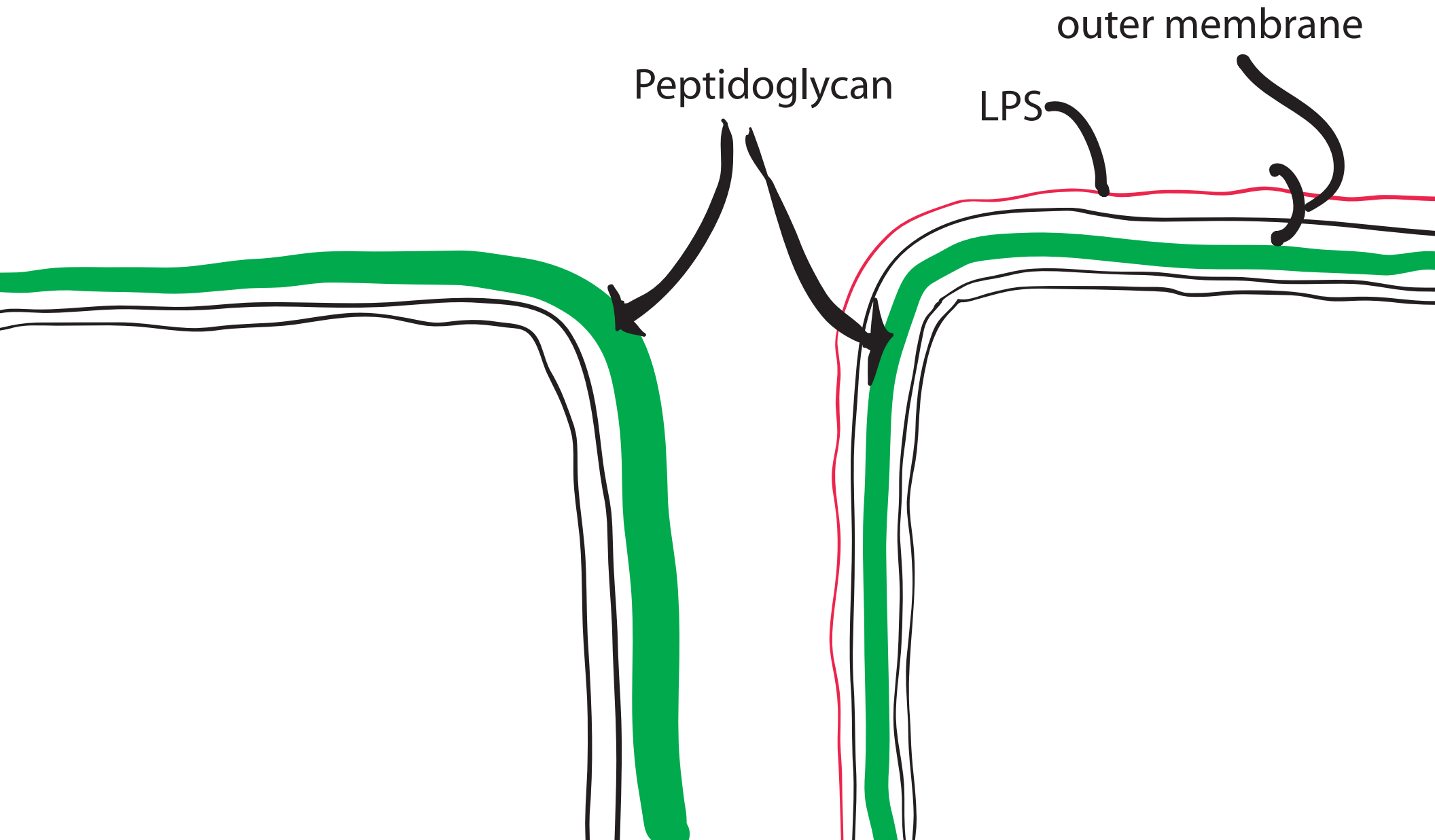


Medzhitov et al 1997 Nature 388:394-7

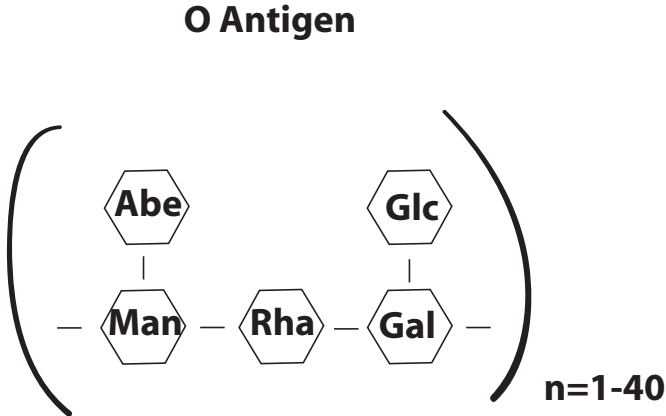
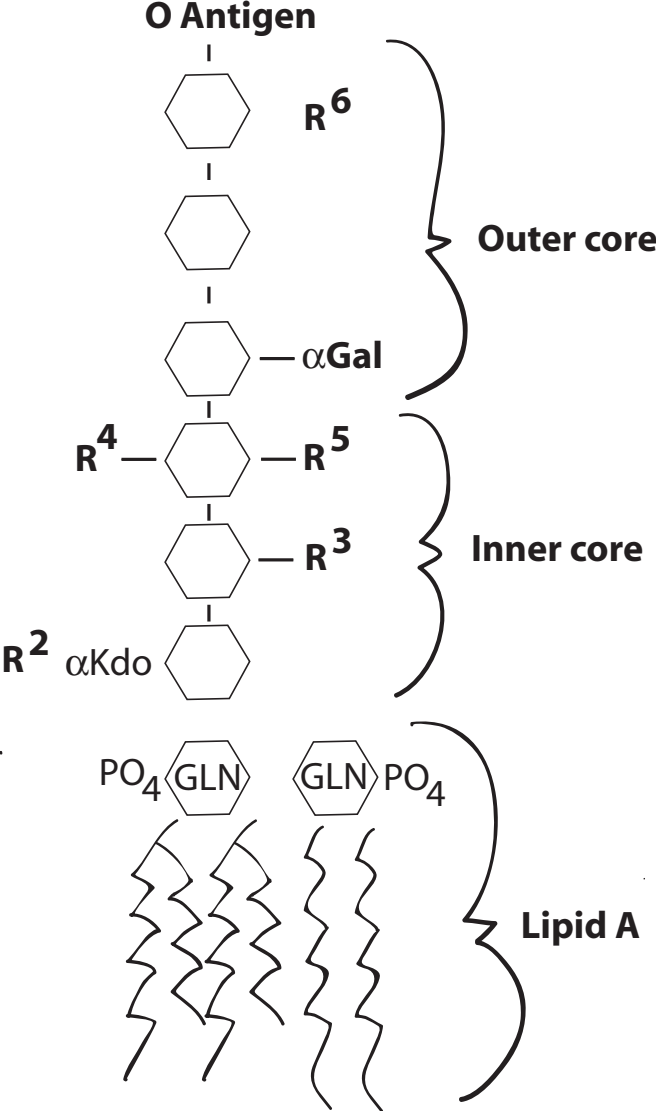
Gram positive and Gram negative bacteria

Gram positive

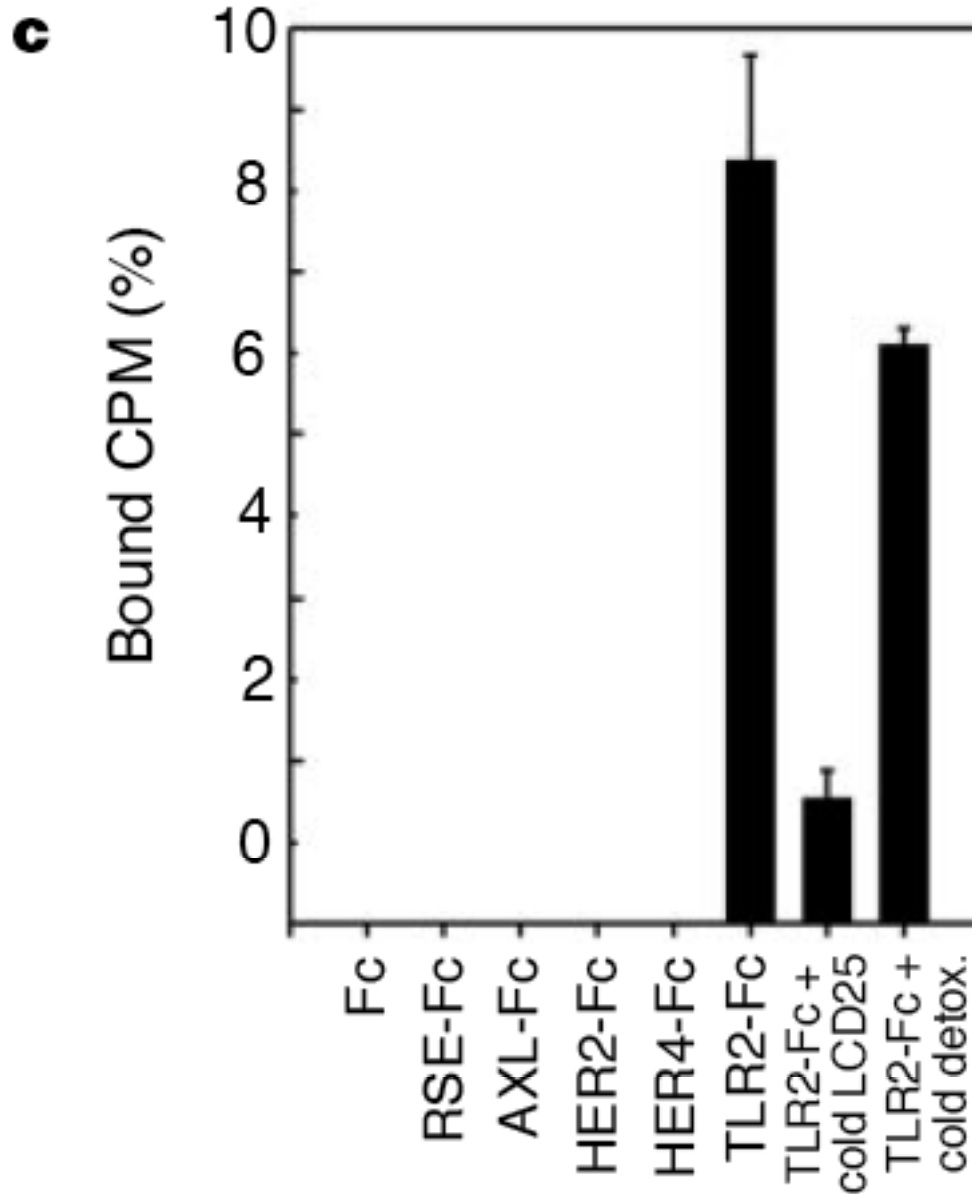
Gram negative



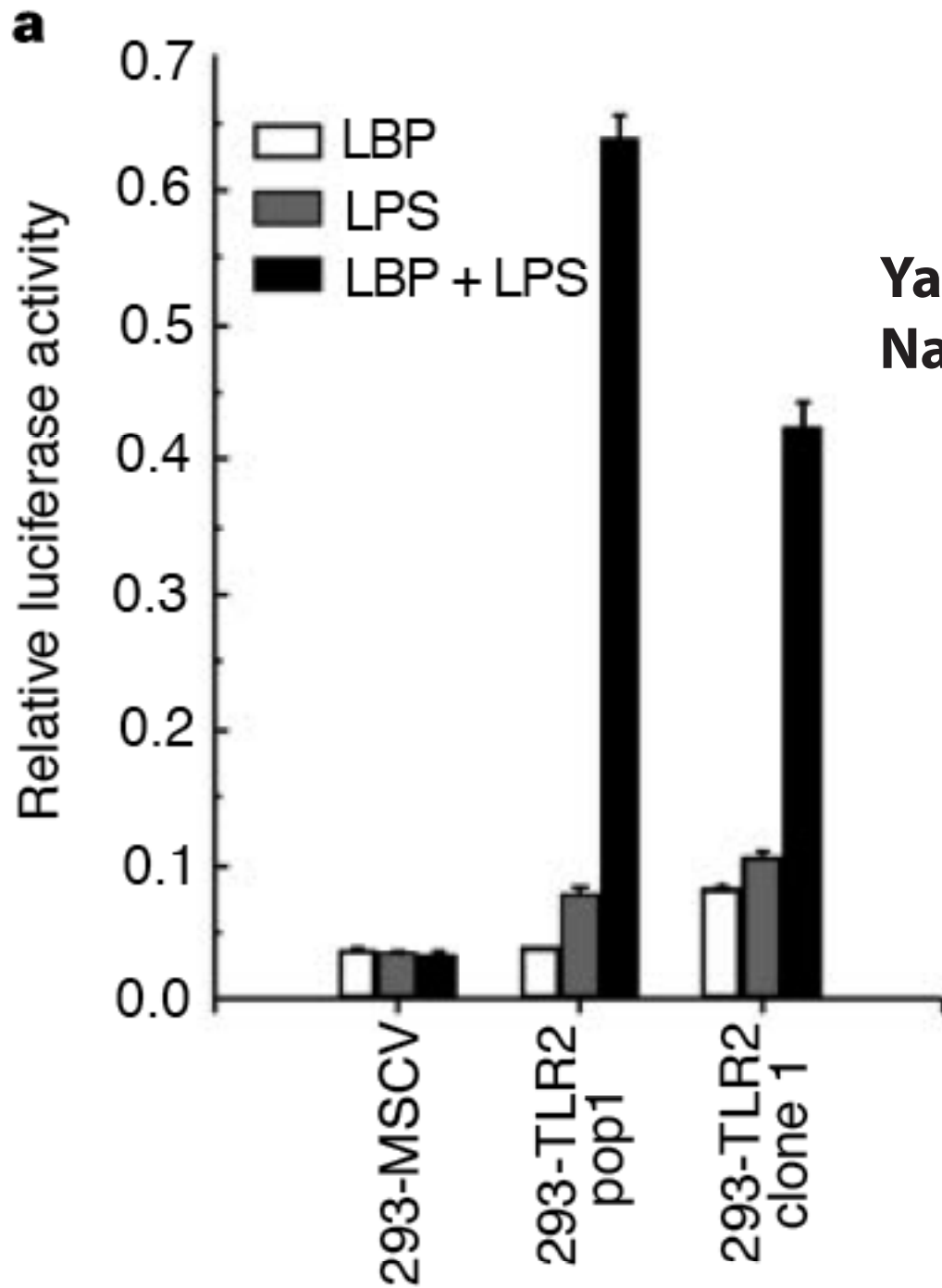
Lipopolysaccharide (LPS) structure



Does Tlr2 really bind to LPS?



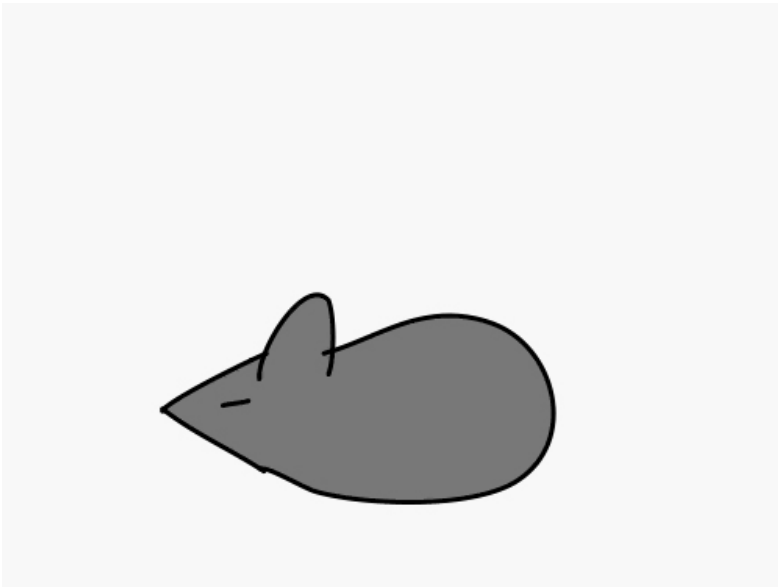
Yang et al. 1998
Nature 395: 284-9



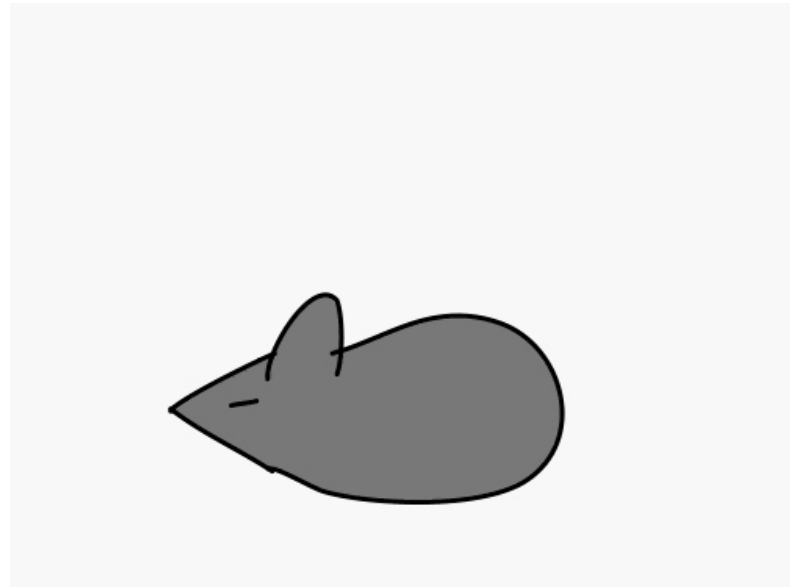
**Yang et al. 1998
Nature 395: 284-9**

Mapping of the LPS mutation identified Tlr4 as a player in LPS signaling

LPS resistant



LPS sensitive



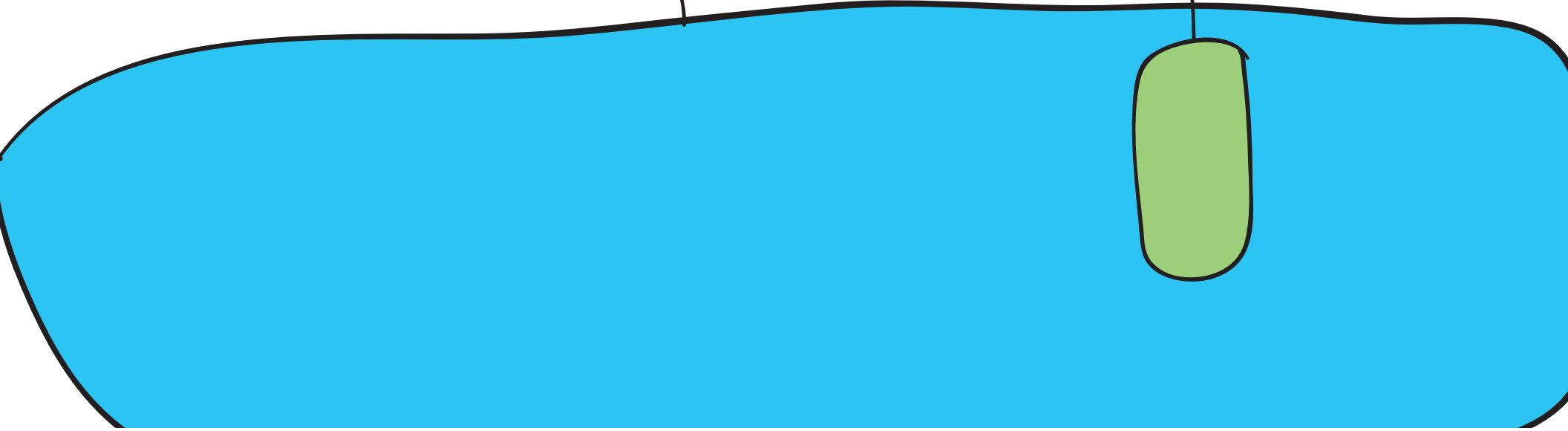
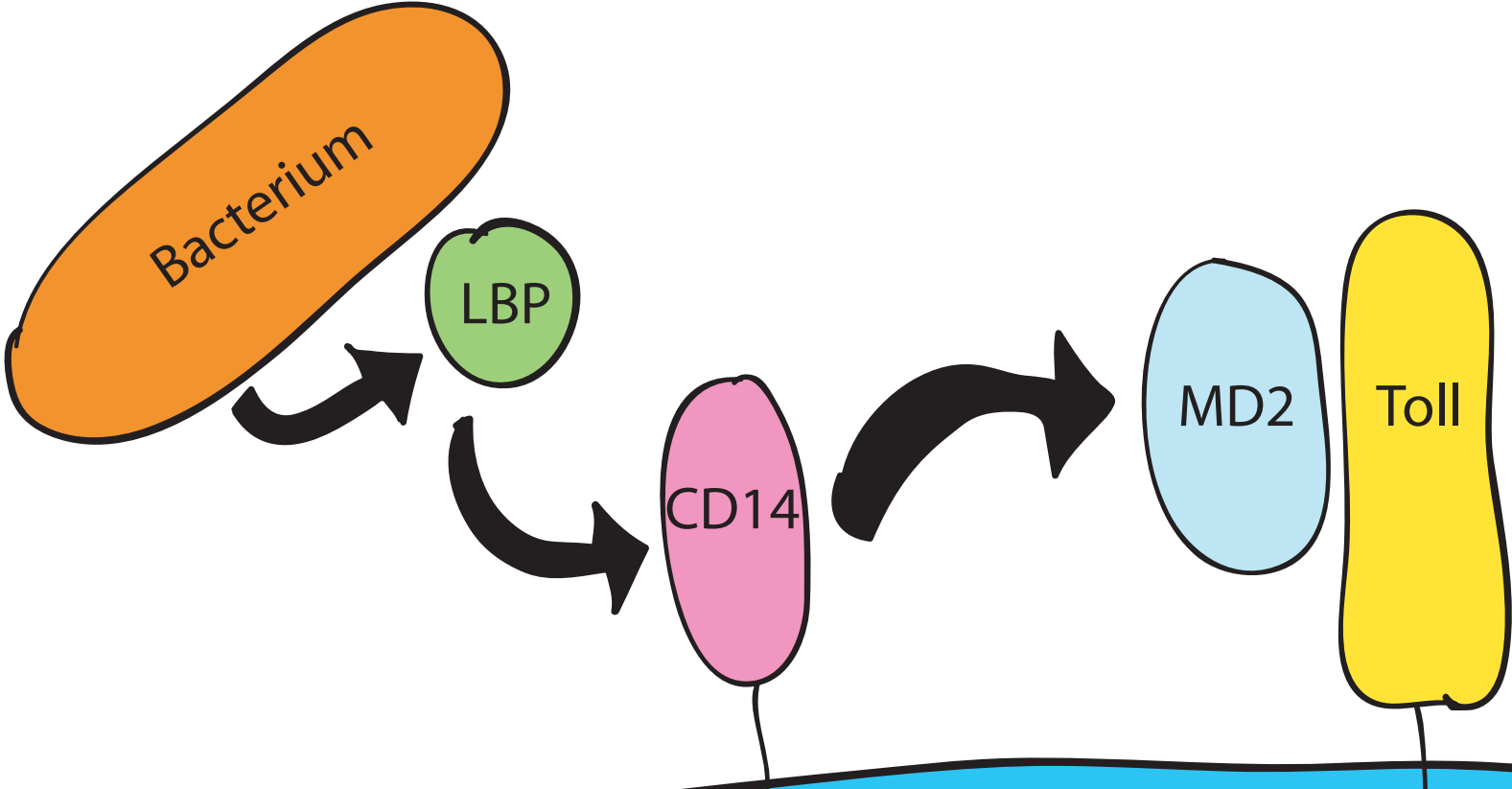
In the mouse Tlr4 mutations cause a dominant loss of response to LPS

		mouse/rat		
		712		
C3H/HeJ (700) ...RFHLCLHYRDFI	H		GVAIAANIIQEGFHKS...(730)	
C3H/HeN(700) ...RFHLCLHYRDFI	P		GVAIAANIIQEGFHKS...(730)	
rat (700) ...RFQLCLHYRDFI	P		GVAIAANIIQEGFHKS...(730)	
human (702) ...PFQLCLHYRDFI	P		GVAIAANIIHEGFHKS...(732)	
● ●			●	

transcript not expressed in C57BL/10ScCr mice either

Poltorak et al. 1998 Science 282: 2085-88

Transfer of LPS



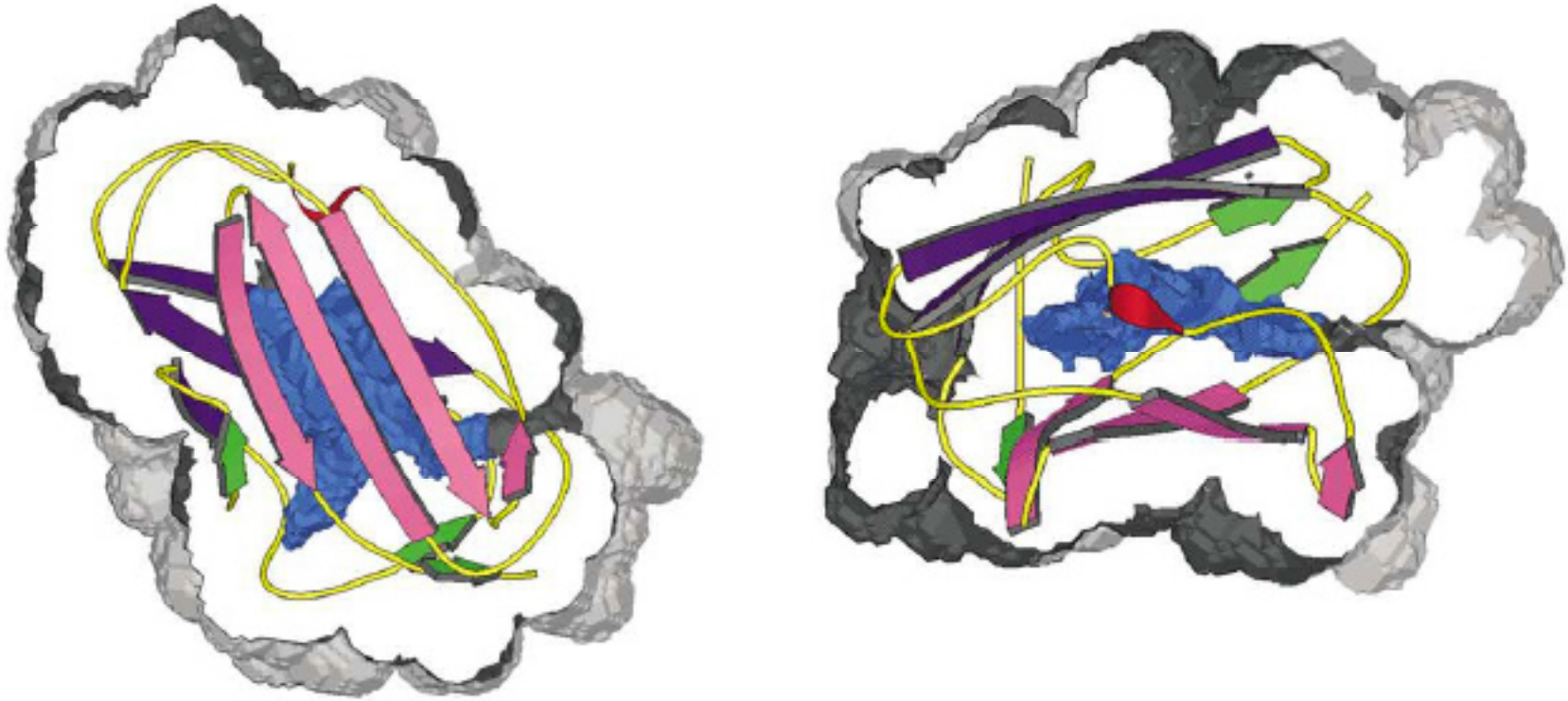
LOS is processed from aggregates and passed to MD2 via CD14

Table 1. Ability of various forms of ¹⁴C-LOS with or without proteins to form LOS:MD-2 and activate HEK/TLR4

Materials	LOS:MD-2	Activation
LOS _{agg} + LBP	---	---
LOS _{agg} + LBP, MD-2	---	---
LOS _{agg} , LBP, sCD14	---	---
LOS _{agg} , LBP, sCD14, MD-2	+++	+++
LOS:sCD14	---	---
LOS:sCD14 + MD-2	+++	+++
LOS:sCD14 + conditioned culture media (no MD-2)	---	---

Gioannini et al. 2004 PNAS101: 4186-4191

Crystal structure of Der P 2



Derewenda et al. 2002 JMB 318:189-197

Tolls are activated by a range of elicitors

Lipopeptide

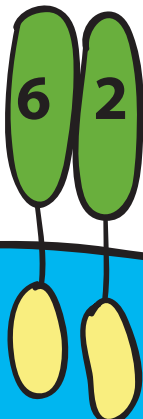
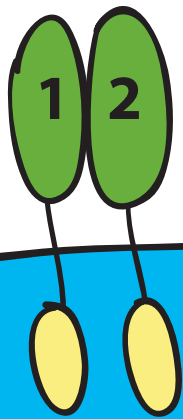
Zymosan
glycolipid
peptidoglycan

LPS
HSP70

Monomeric
flagellin

?

Uropathogenic
E.coli



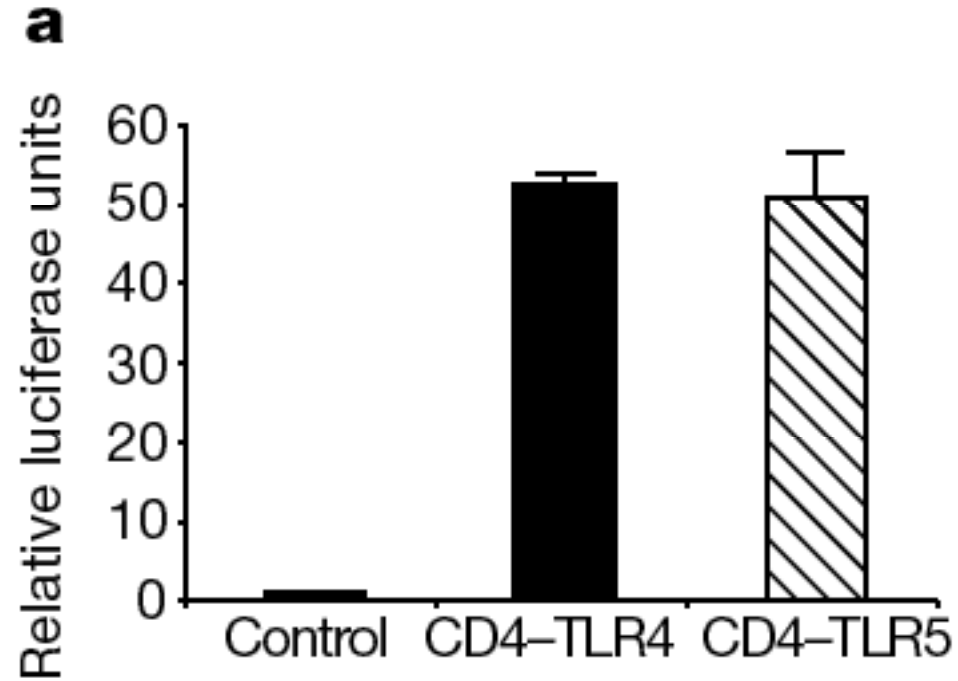
dsRNA

ssRNA

CpG

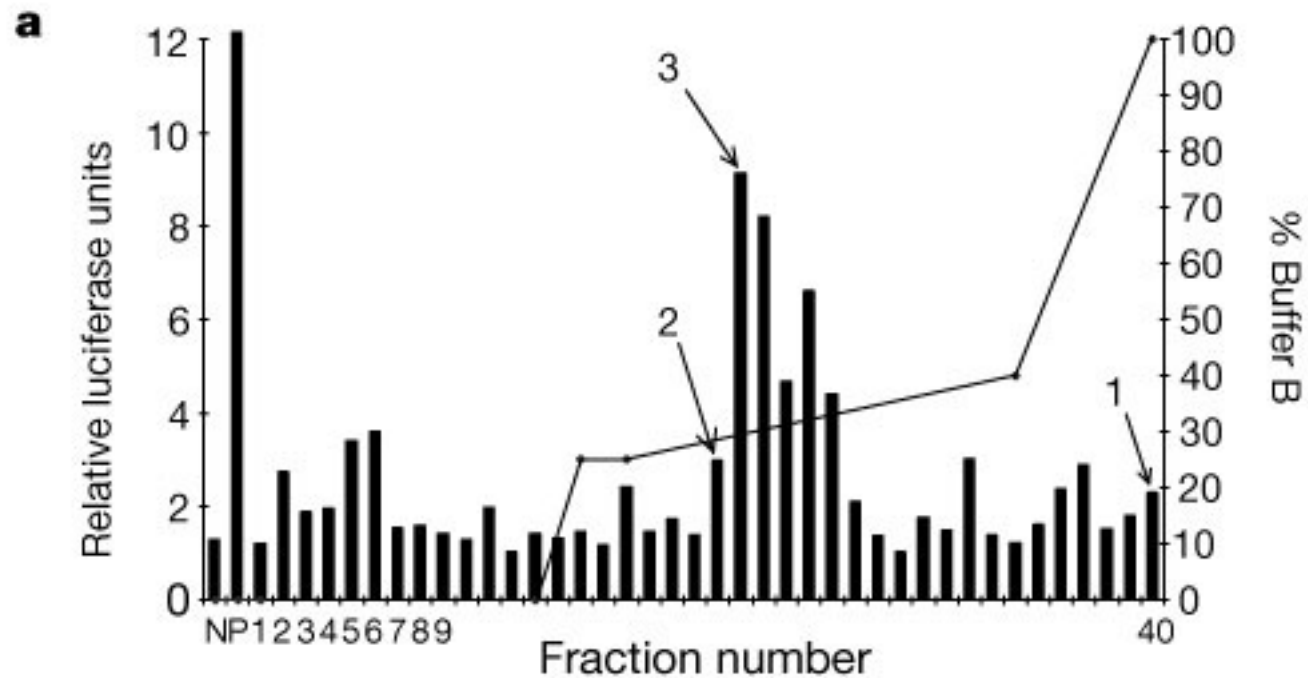


Activated Tlr5 induces NFkB



Hayashi et al. 2001 Nature 410: 1099-1103

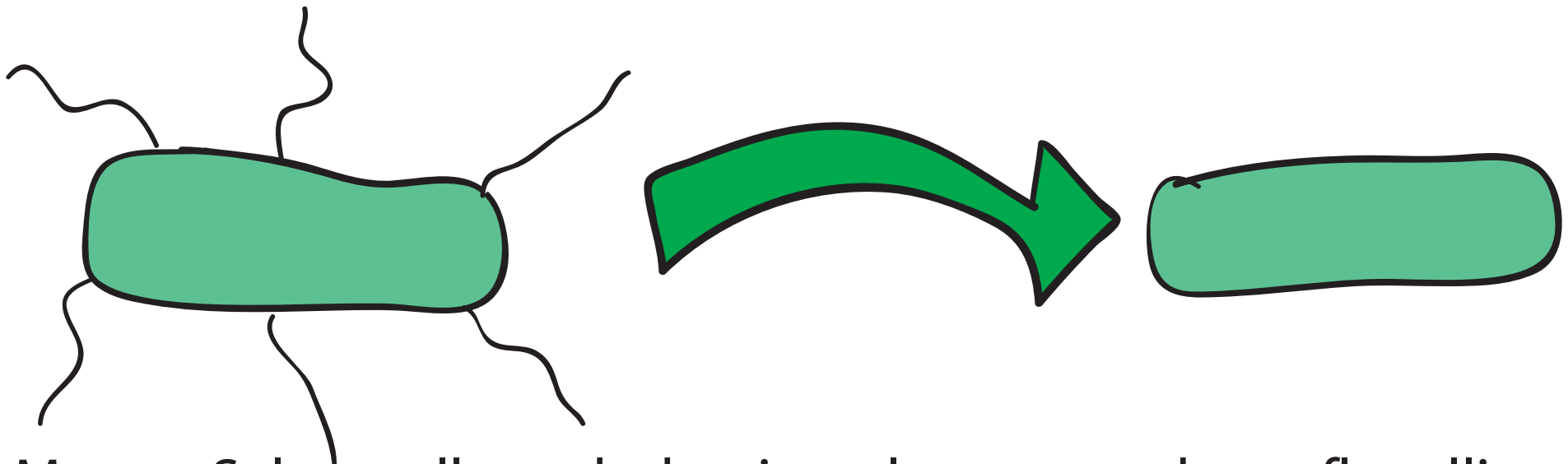
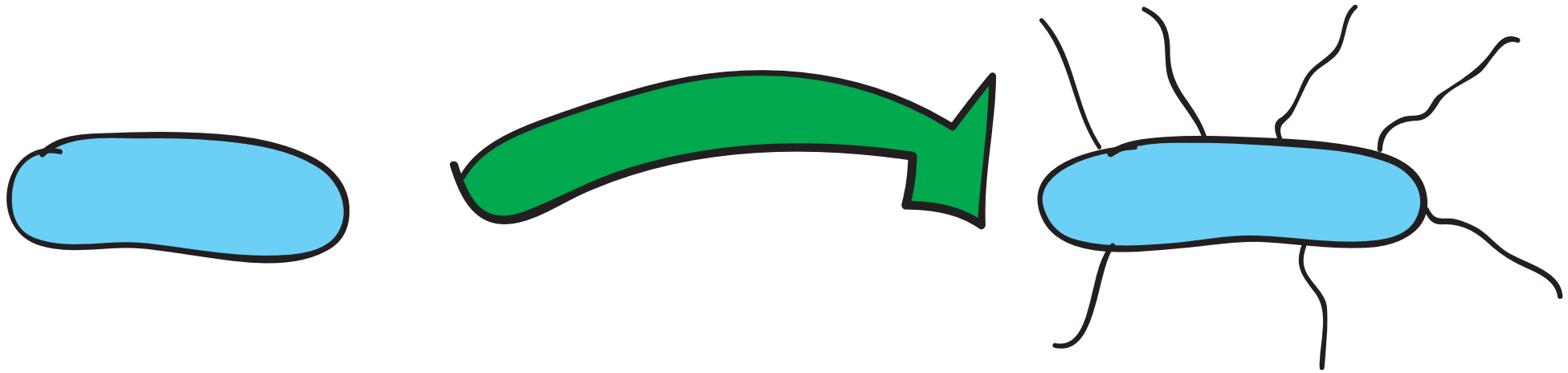
Purification of the PAMP signaling through TLR5



Hayashi et al. 2001 Nature 410: 1099-1103

Testing the role of flagellin in Tlr5 induction

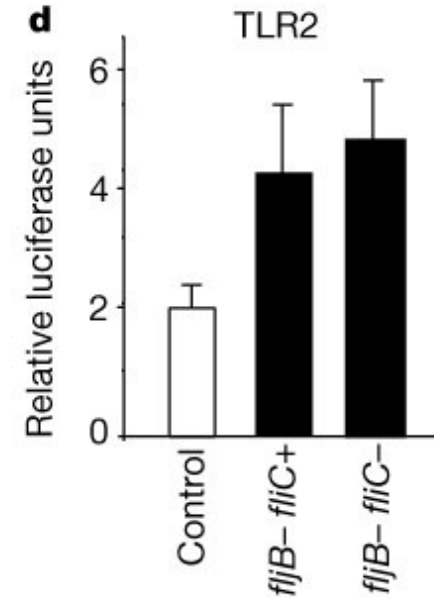
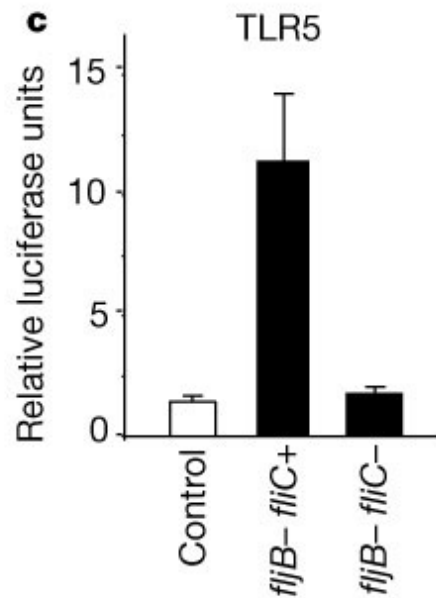
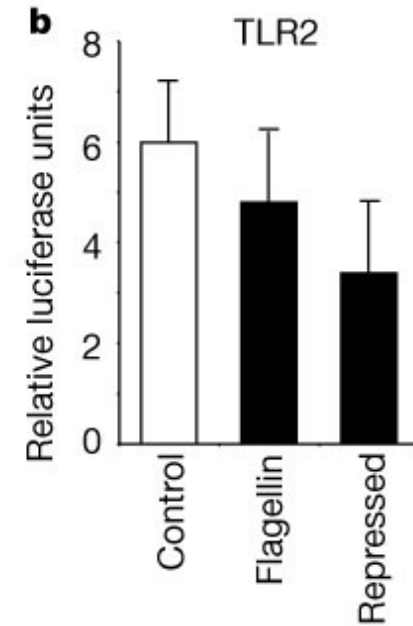
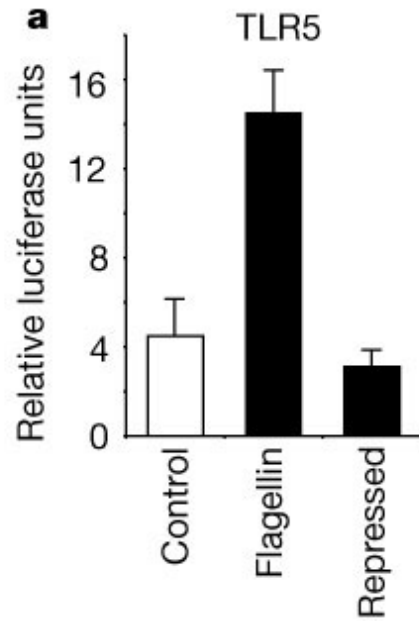
Induce non-flagellated E.coli to express Listeria flagellin



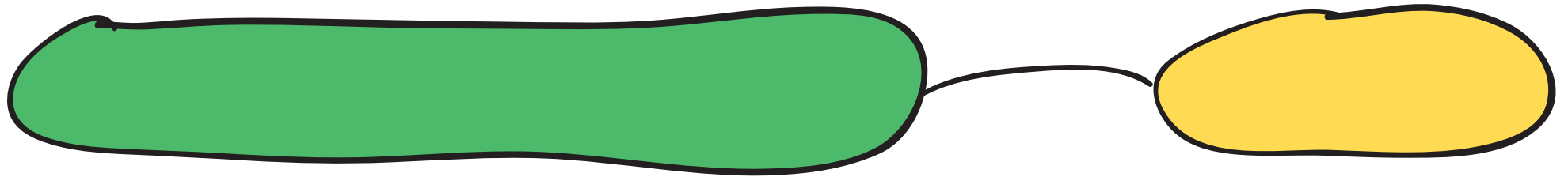
Mutate Salmonella such that it no longer produces flagellin

Flagellin signaling

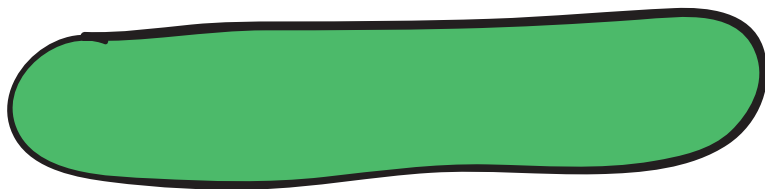
Hayashi et al. 2001
Nature 410: 1099-
1103



A common allele of Tlr5 encodes a stop codon



Wild type Tlr5

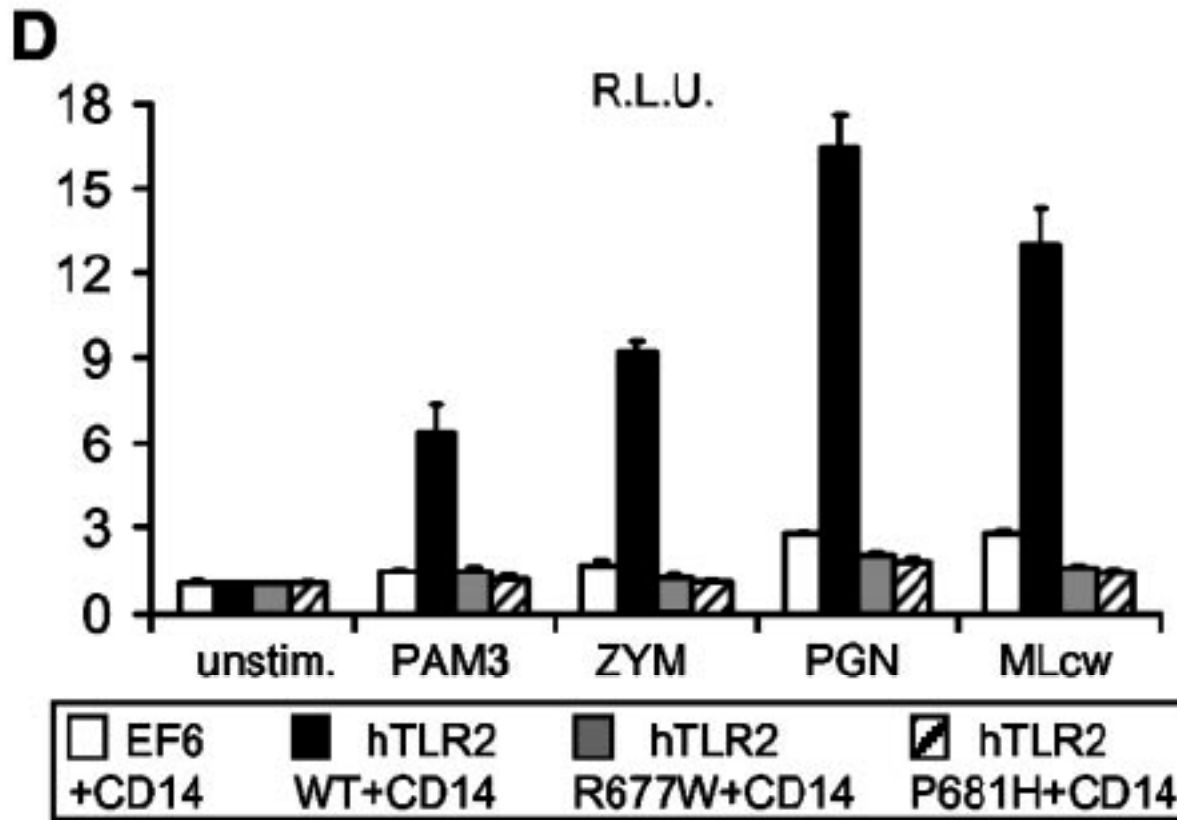


Tlr5^{392STOP}

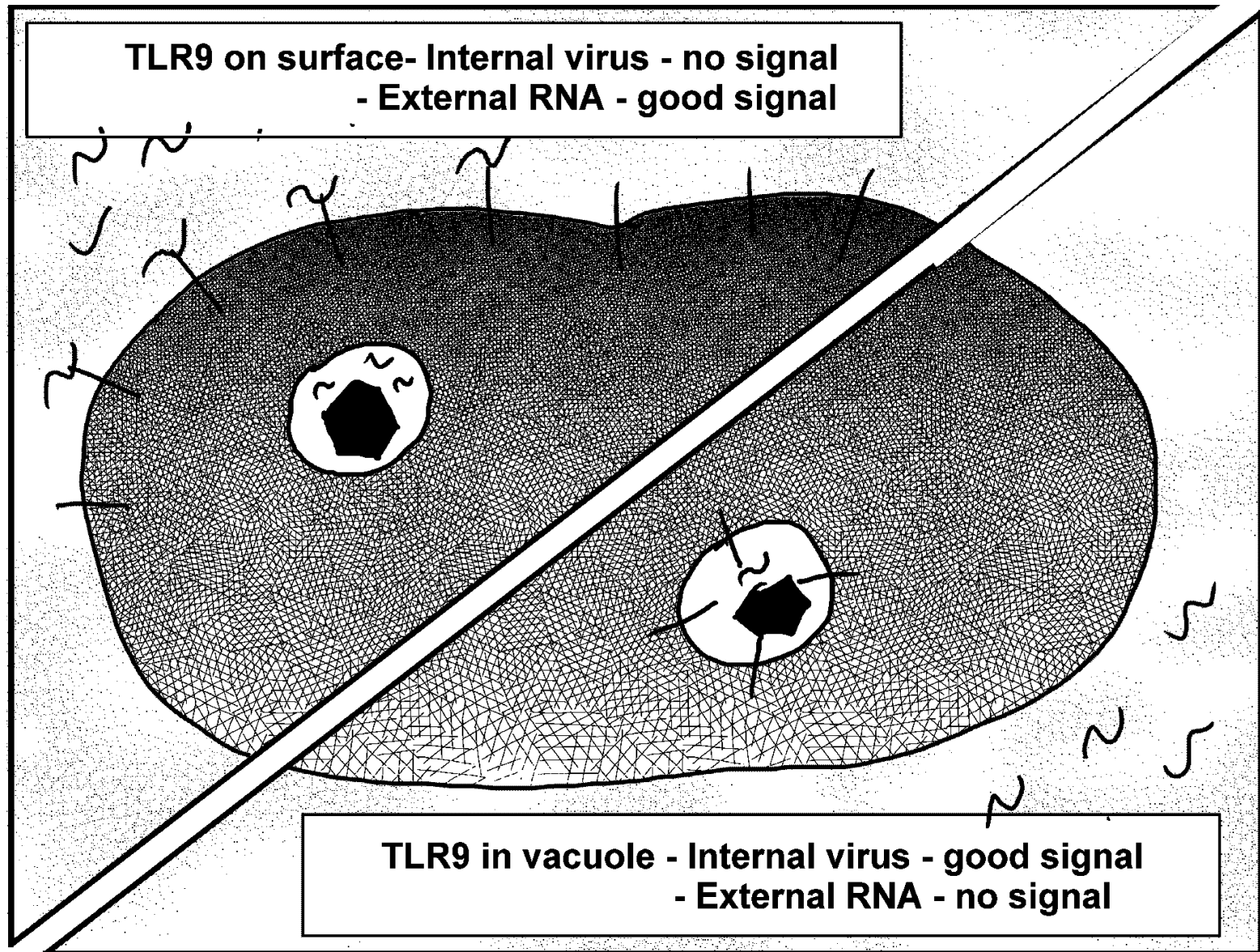
Hawn et al 2003 J. Exp. Med. 198: 1563-1572

Tlr2 mutations predispose a patient towards lepromatous rather than tubercular leprosy

Arg677 -Trp mutation



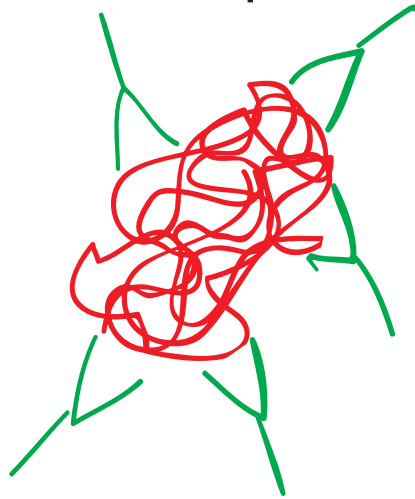
The location of the Tlr affects its function



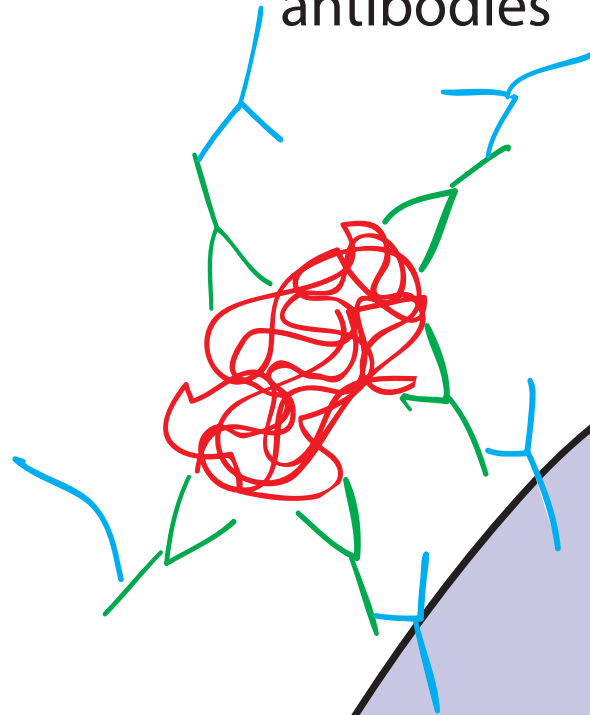
Nat Immunol. 2006 Jan;7(1):49-56

A role for Tlr9 in Lupus

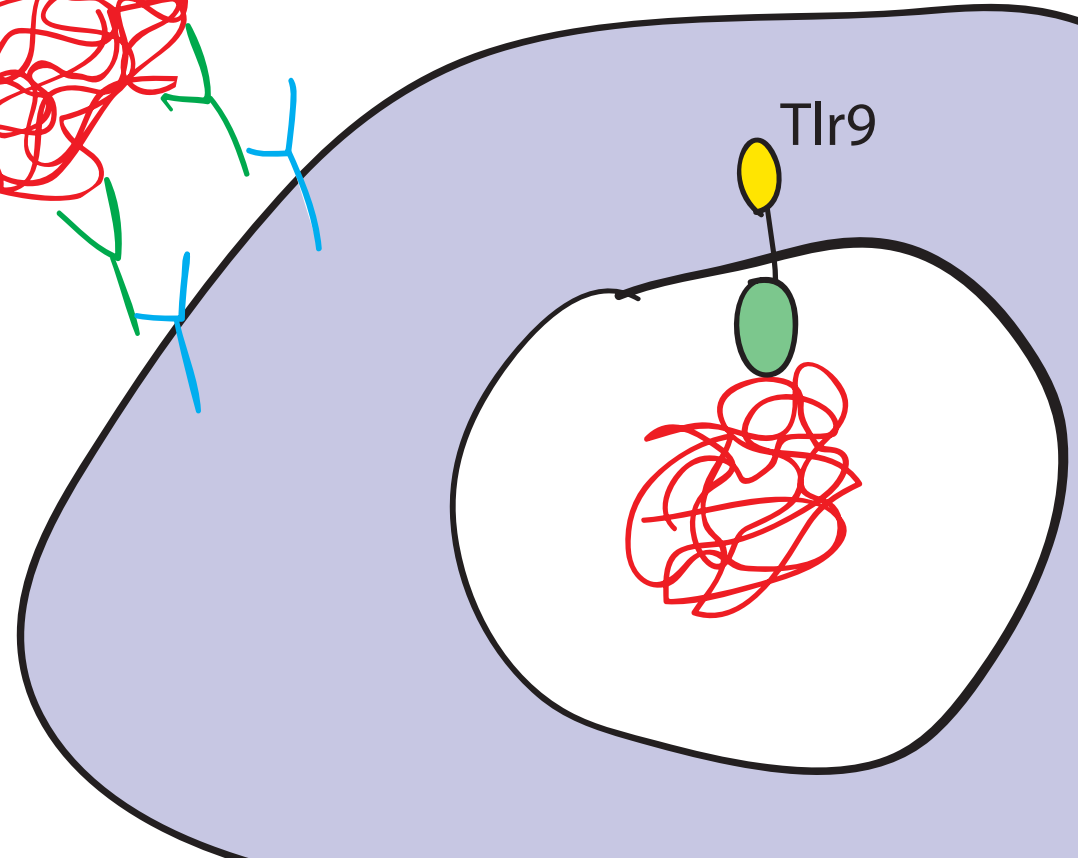
Chromatin-antibody
complex



anti-iGg2a
antibodies



Tlr9



Leadbetter et al. 2002 Nature 416: 603-607

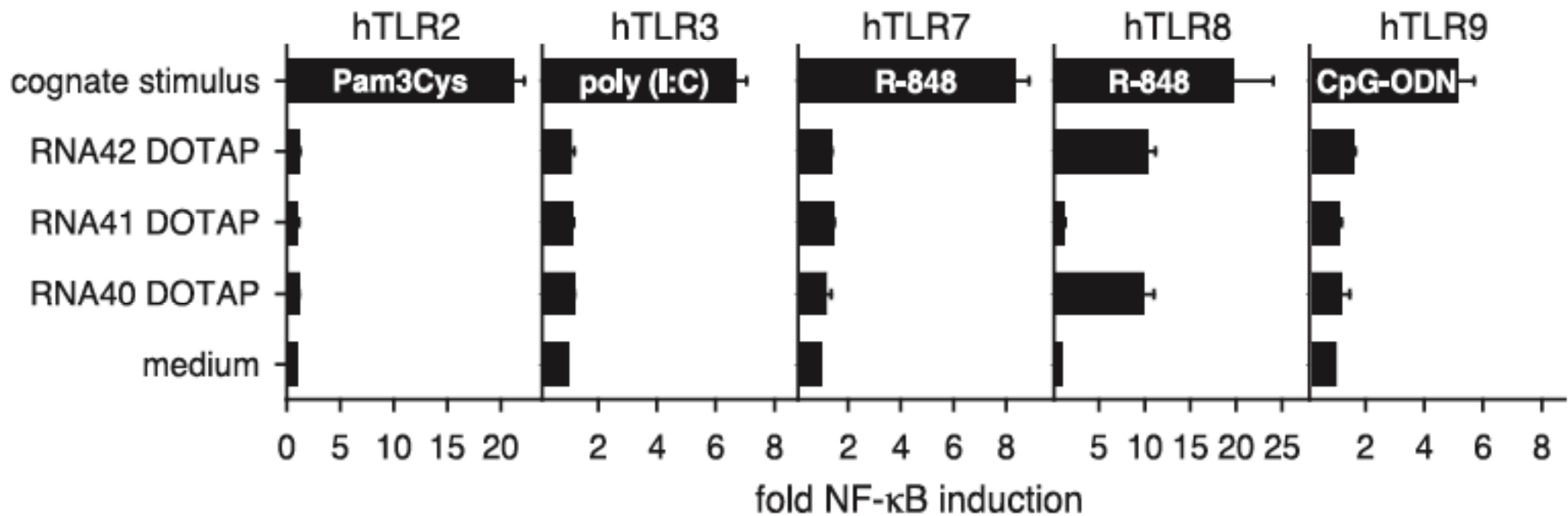
Drugs and adjuvants based on toll signaling

Company	Product	Use	Mechanism
3M	imiquimod	genital warts	Tlr7 agonist
Coley	synthetic CpG oligo	lymphoma	Tlr9 agonist
Dynavax	1018 SS	Hep B vaccine	Tlr9 agonist
Idera	IMOxine	carcinoma	Tlr9 agonist
Eisai	E5564	Sepsis	Tlr4 antagonist
Anadys	isatoribine	Chronic HepC	Tlr7 agonist

Nature Biotechnology

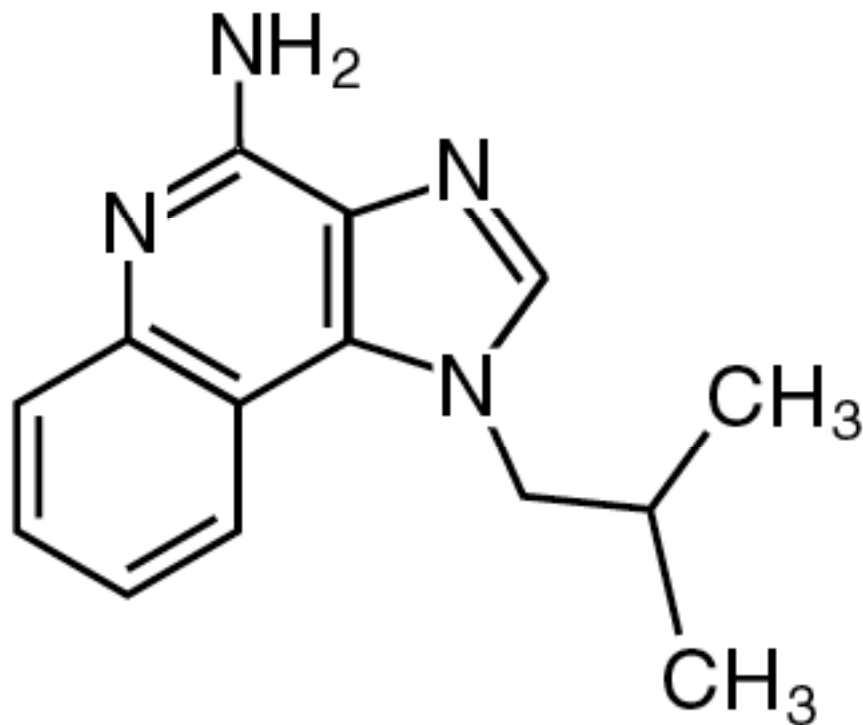
Published online: 6 March 2006; | doi:10.1038/nbt0306-230

A natural ligand of Tlr7/8 is ssRNA



Heil et al. 2004 Science 303: 1526-1529

Imiquimod - sold as Aldara



Tlr 7/8 agonist with potent antiviral activity

Treatment of basal cell carcinoma with imiquimod



Before

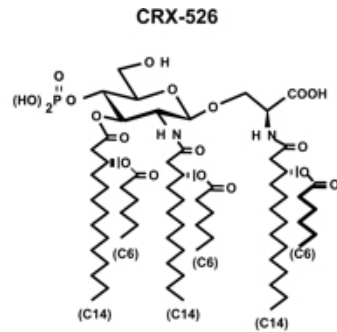
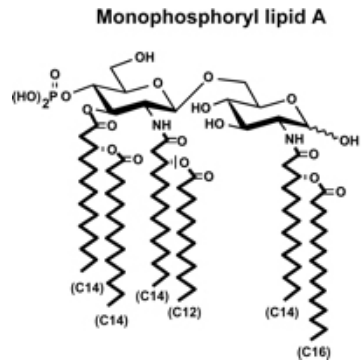


During



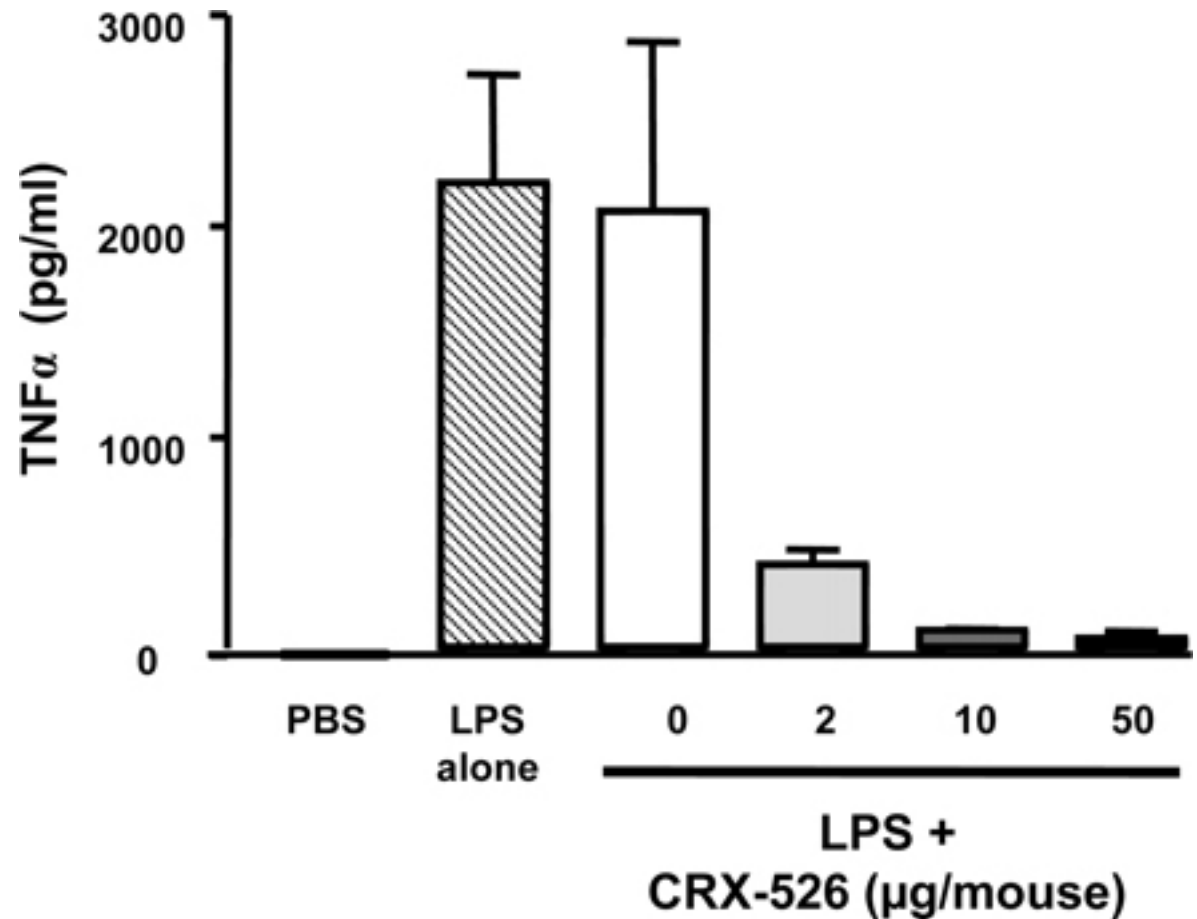
After

Corixa has studied a Tlr4 antagonist



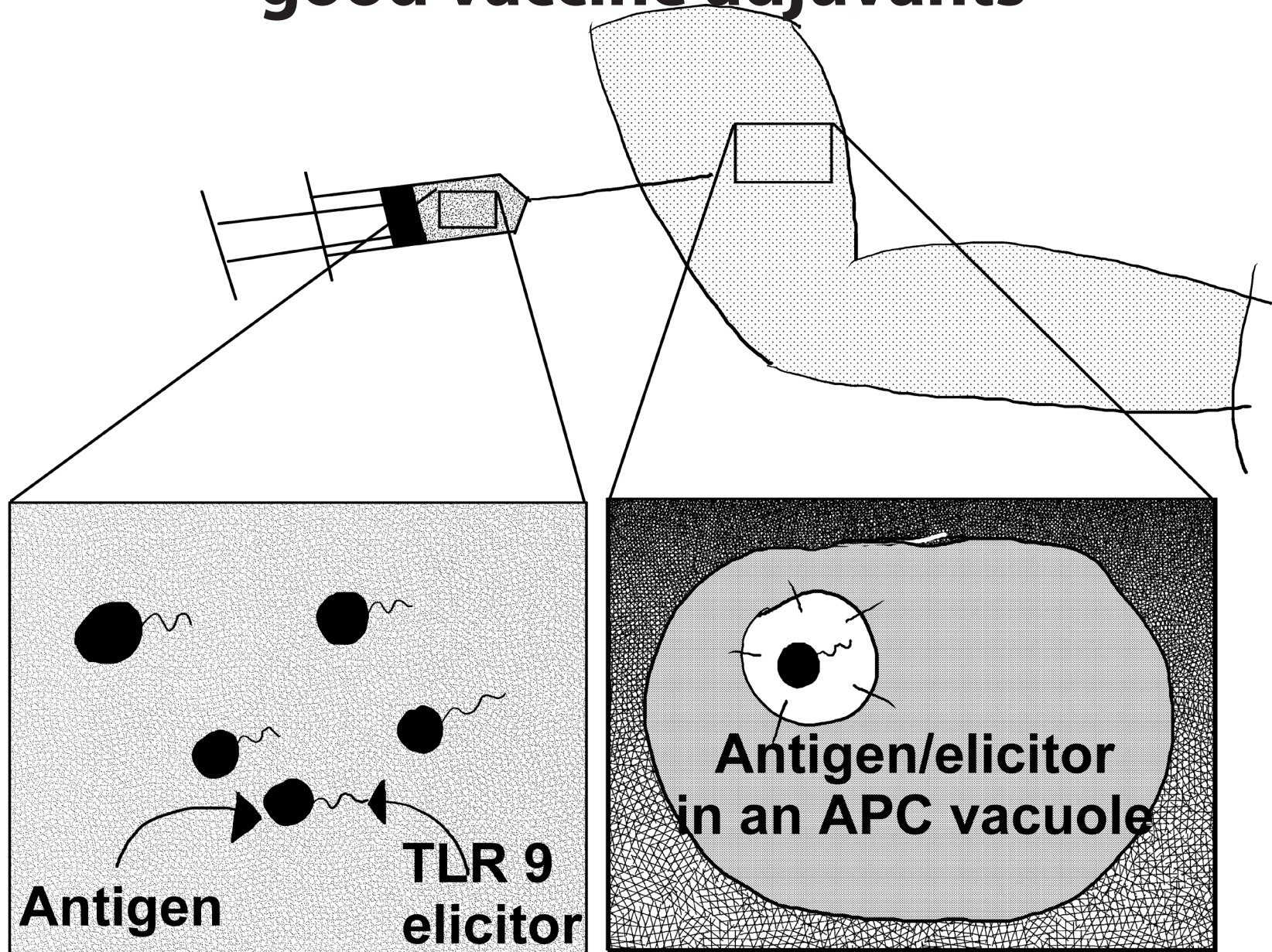
Lipid A

Analogue

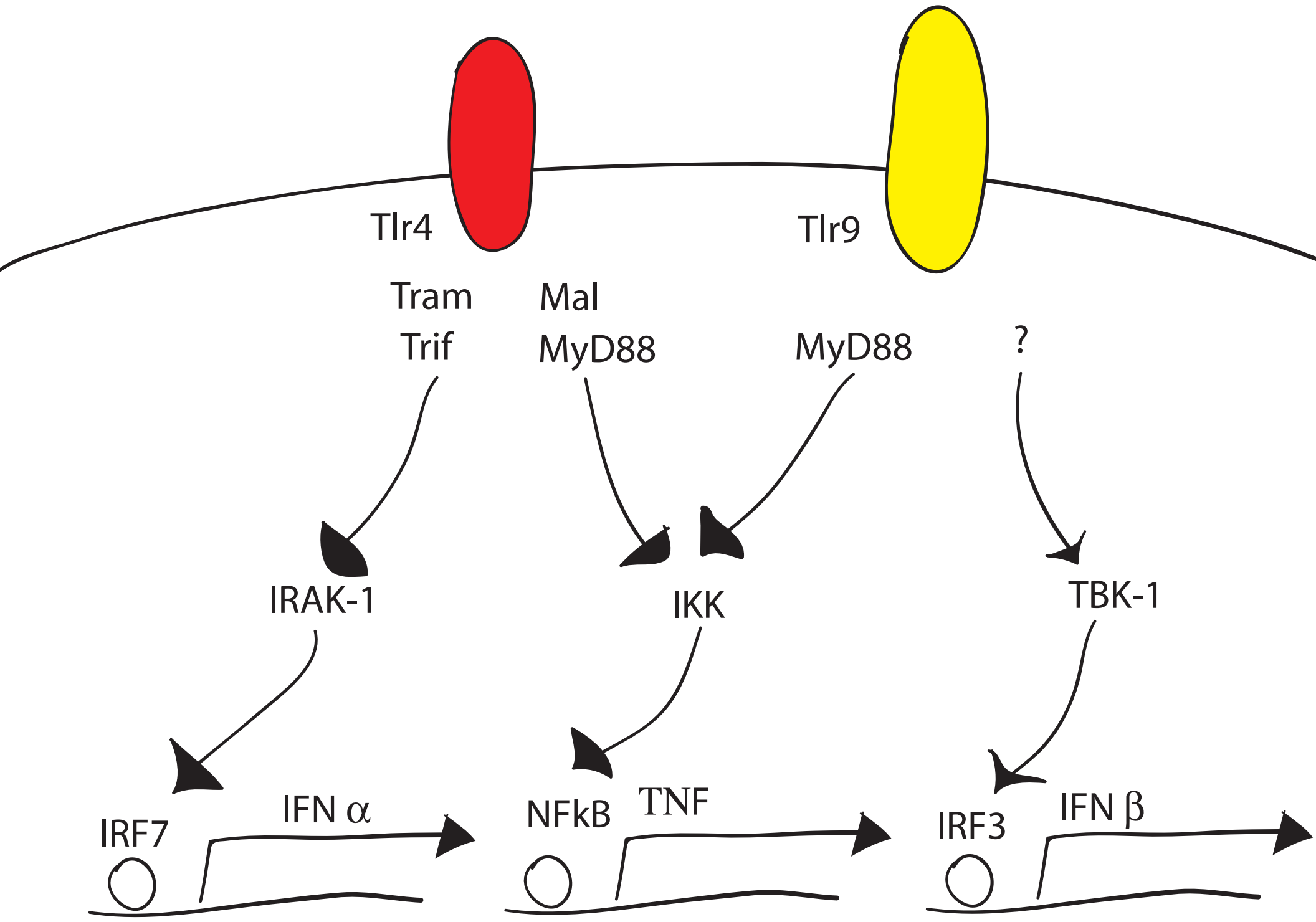


Fort et al. The Journal of Immunology, 2005, 174: 6416-6423.

Toll ligands might make good vaccine adjuvants

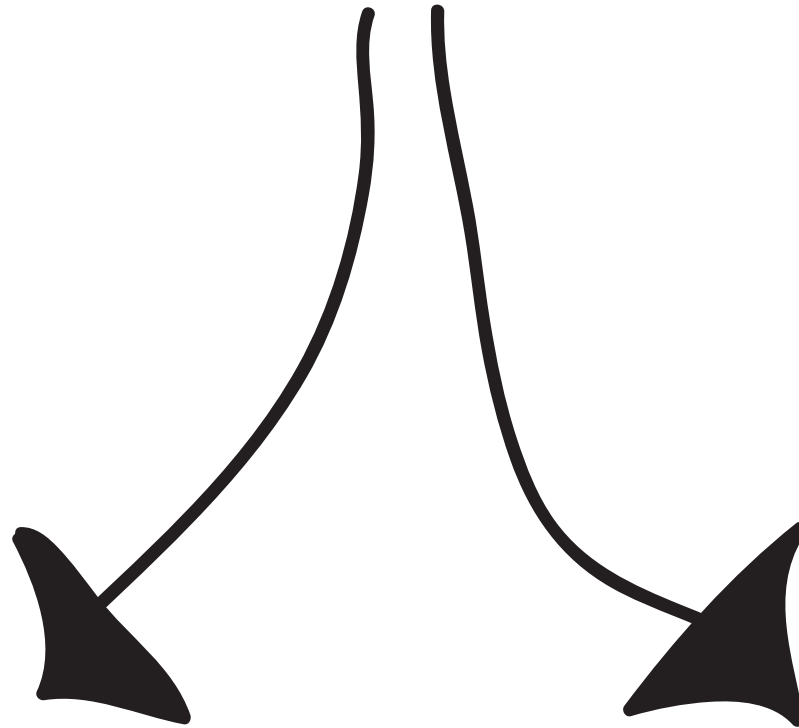


Tlrs signal in different manners



Tlr signaling can skew the Th1/Th2 balance

**Developmental History
Stimulus**

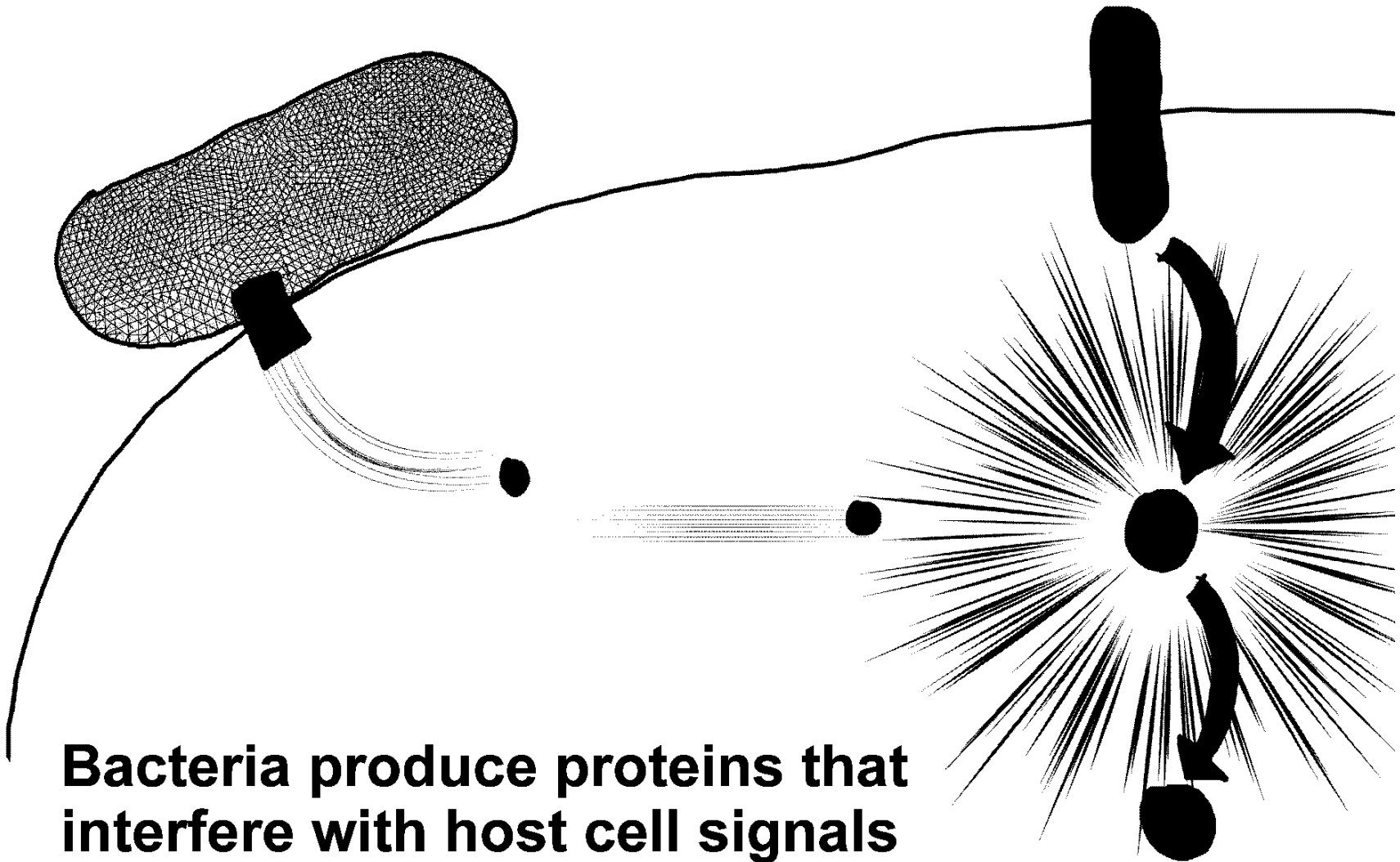


Th1 response

- Cell mediated immunity
- Good for fighting intracellular infections
- Delayed type hypersensitivity

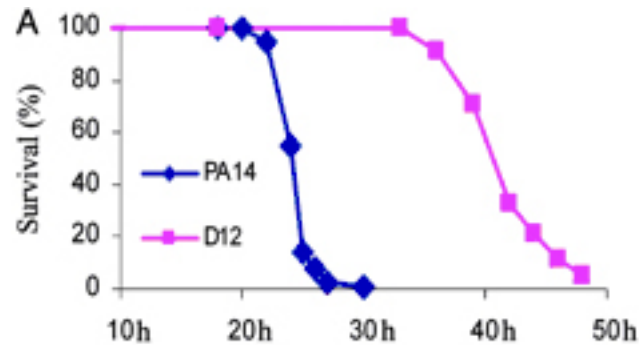
Th2 response

- Antibody mediated immunity
- Good for fighting extracellular parasites
- Allergy

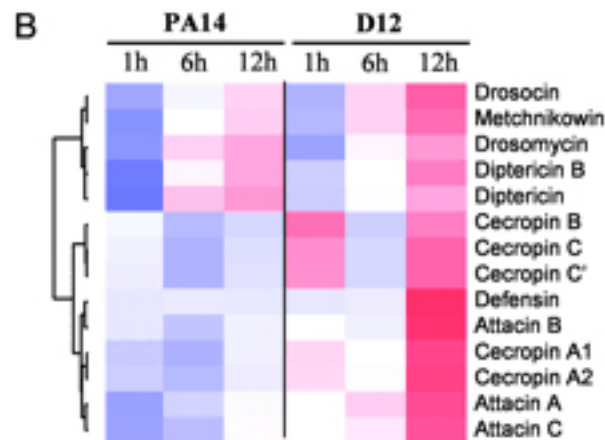


Bacteria produce proteins that interfere with host cell signals

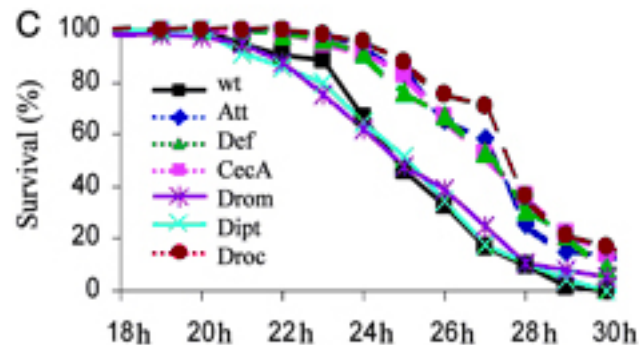
Microbes have evolved methods of inhibiting Toll signaling



Pseudomonas strain PA14 kills better than strain D12

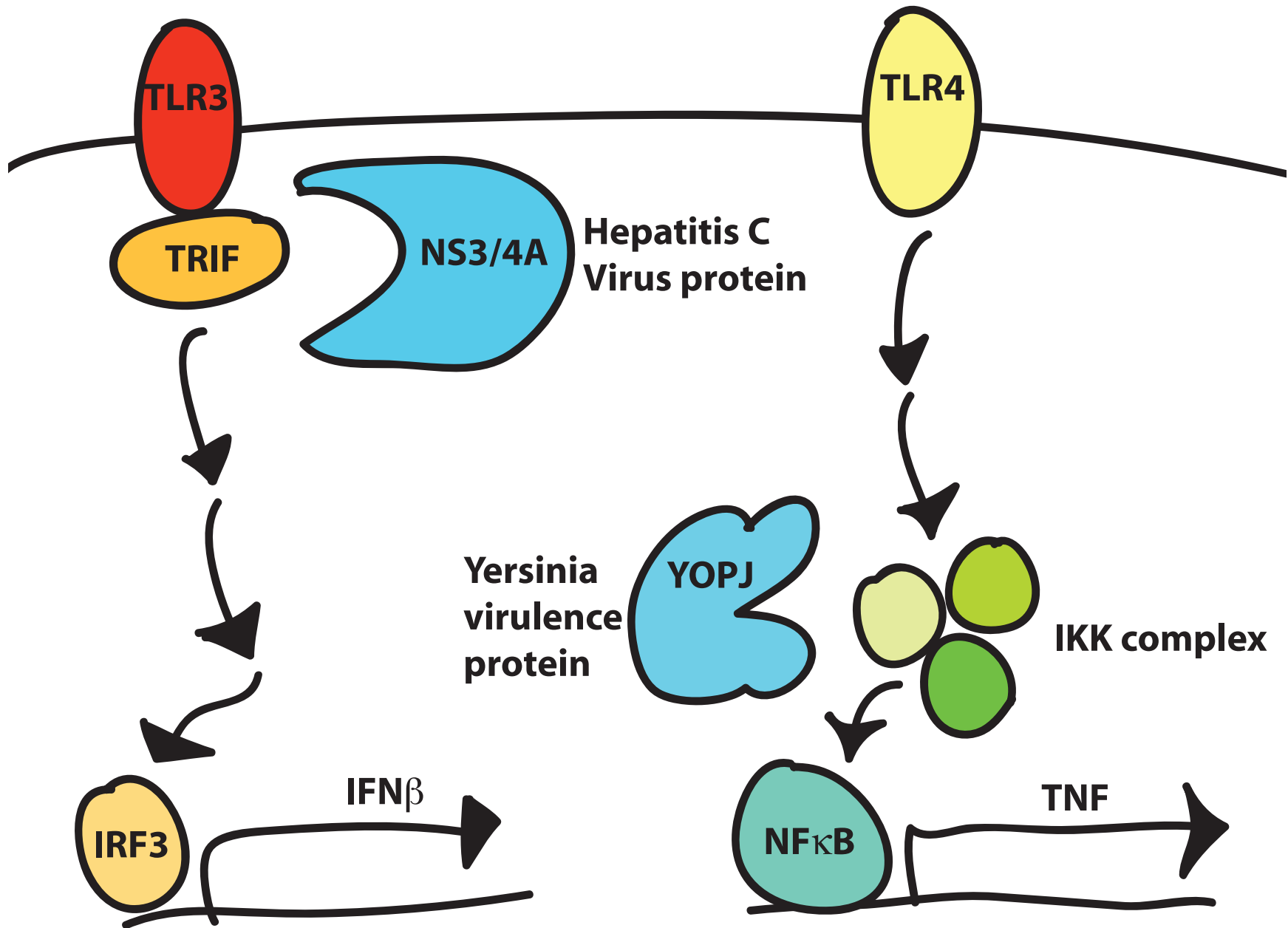


Toll signaling is induced to a lesser degree with PA14 than D12

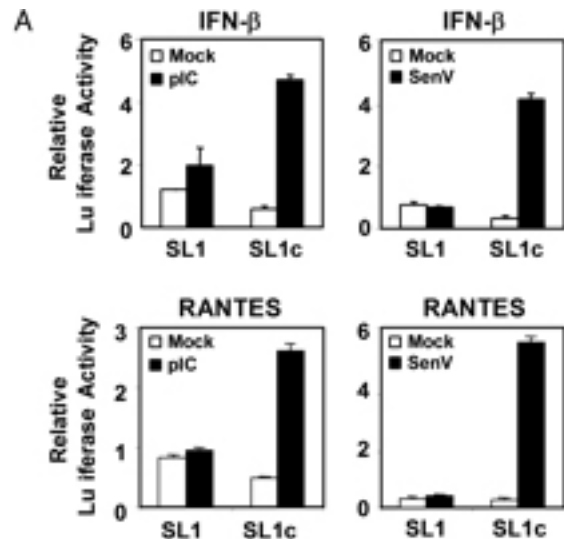


Proc Natl Acad Sci U S A. 2005 Feb 15;102(7):2573-8

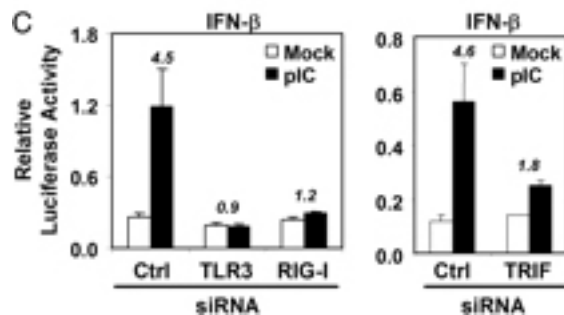
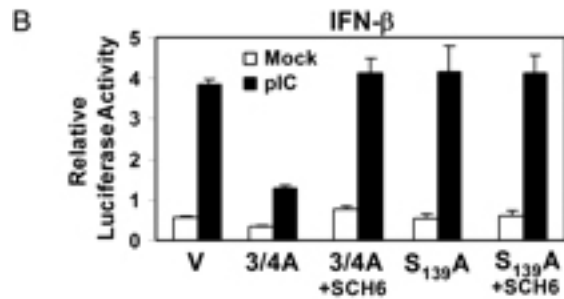
Blocking Toll signaling by viruses and bacteria



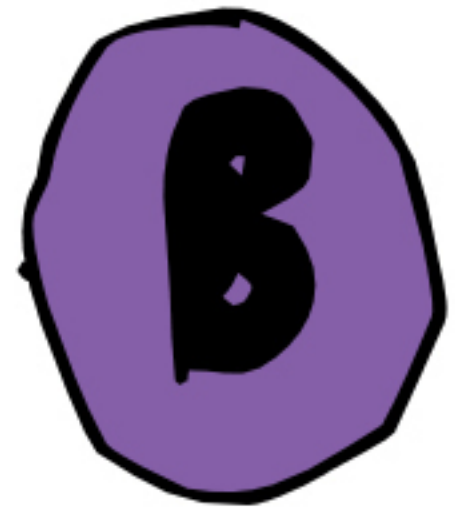
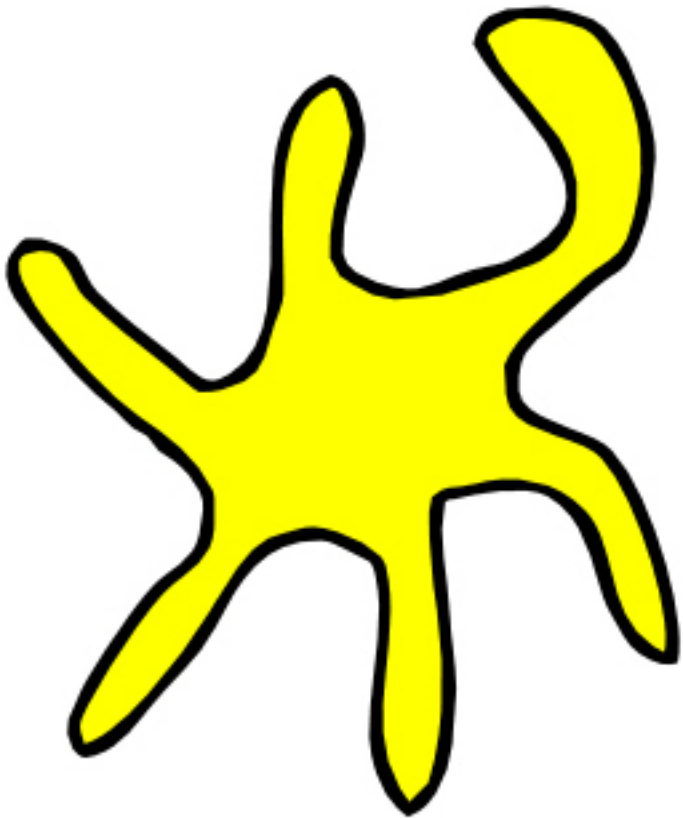
Hepatitis C virus interferes with Toll Signaling



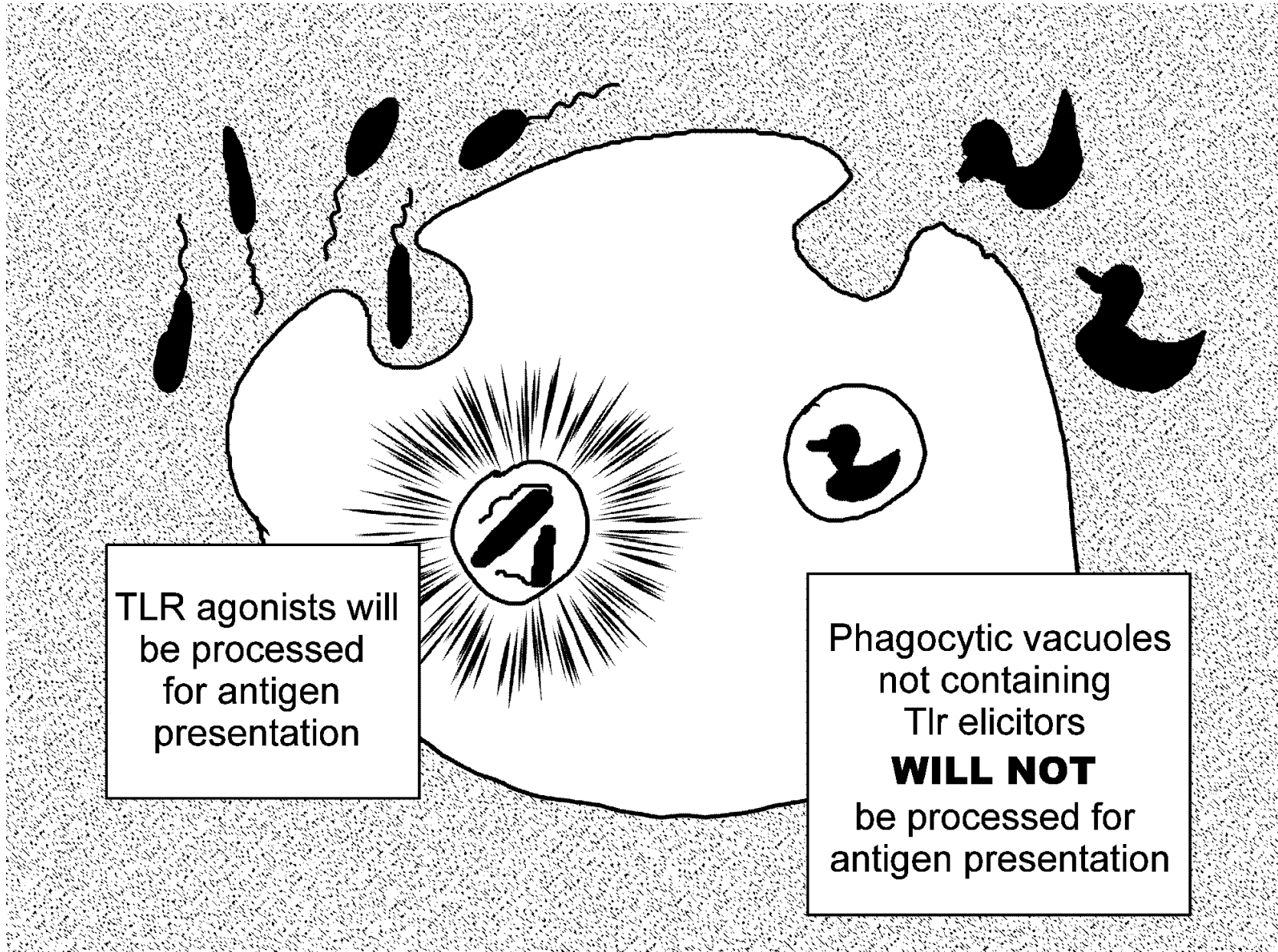
Protease insensitive Trif maintains the Tlr3 response to poly IC



**Clearly, activation of dendritic cells
is important at a whole cell level**



Different types of phagocytosis follow Toll activation.



Nature. 2006 Apr 6;440(7085):808-12

Complement can be activated through three pathways

Classical pathway

Antigen-antibody complexes

C1q, C1r, C1s, C4, C2

Mannan-binding lectin

Mannose on pathogens

MBL, MASP1,2, C4, C2

Alternative pathway

Foreign surfaces

C3, B, D



C3 convertase

**Inflammation
Mediators**

C3a, C5a

Opsonization

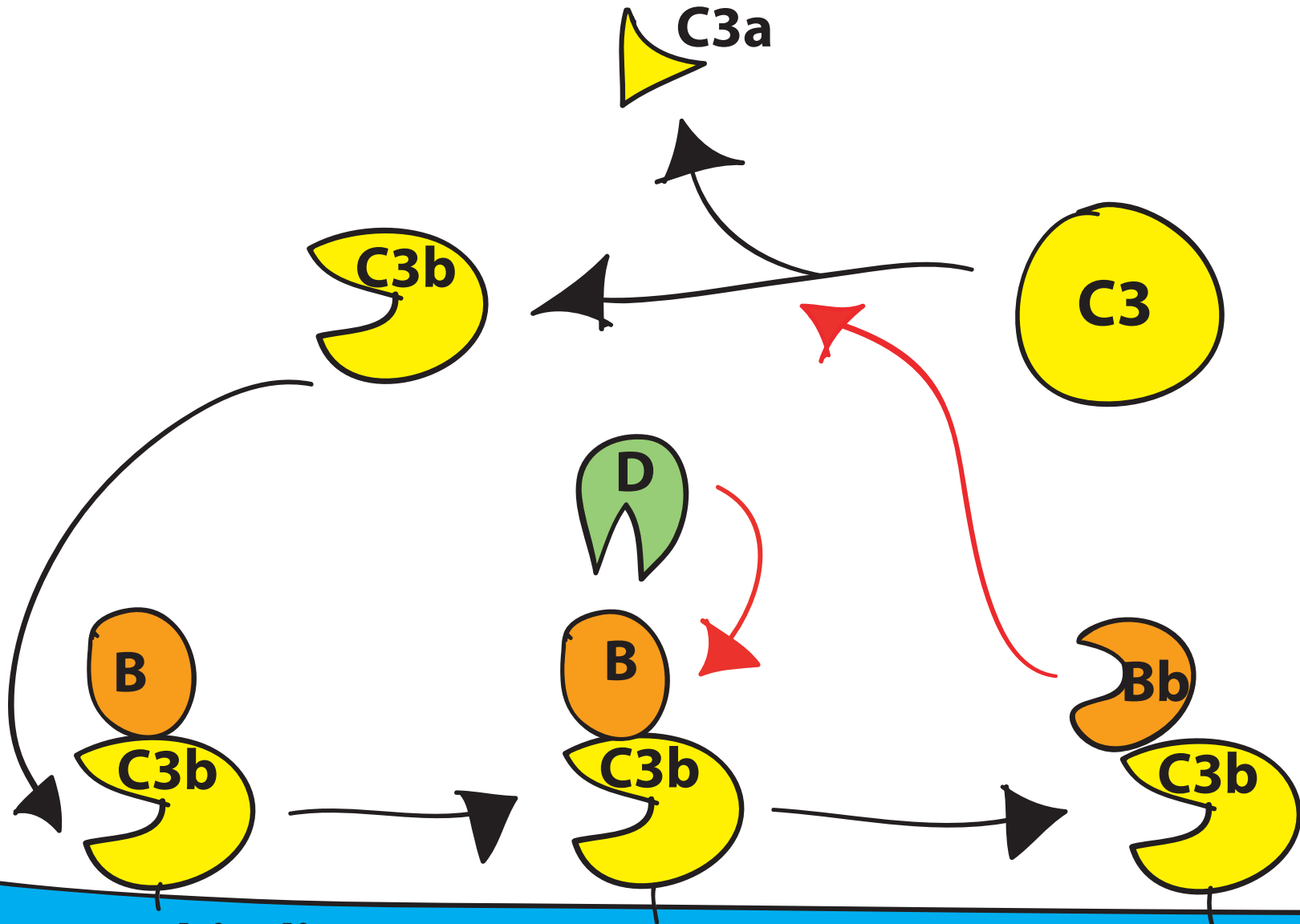
C3b

**Membrane-attack
Complex**

C5b, C6, C7, C8, C9



The alternative pathway

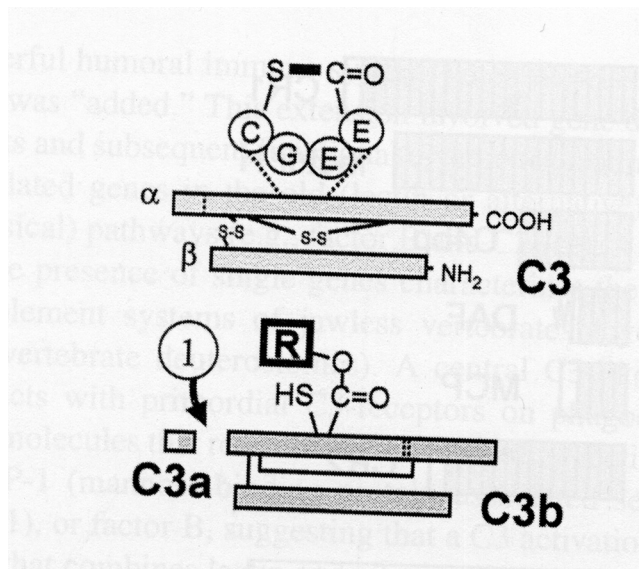


Factor B binding

D cleaves B

C3 convertase

Activation of complement proteases

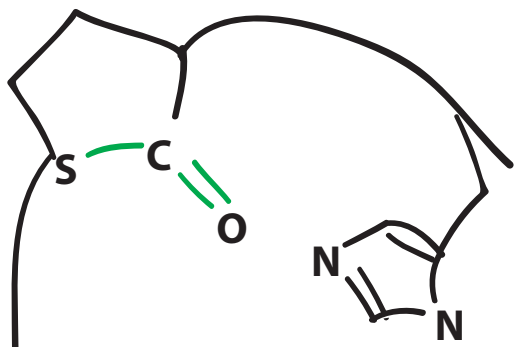


Proteolytic removal of pre-peptide leads to protease activation

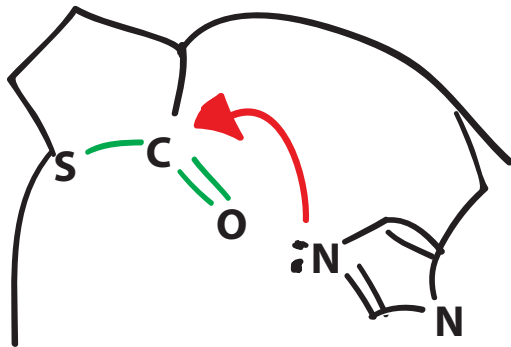
Paul, Vth edition

The thioester bond forms an unstable intermediate

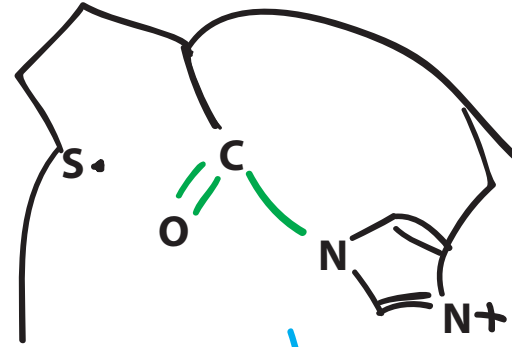
Initial thioester



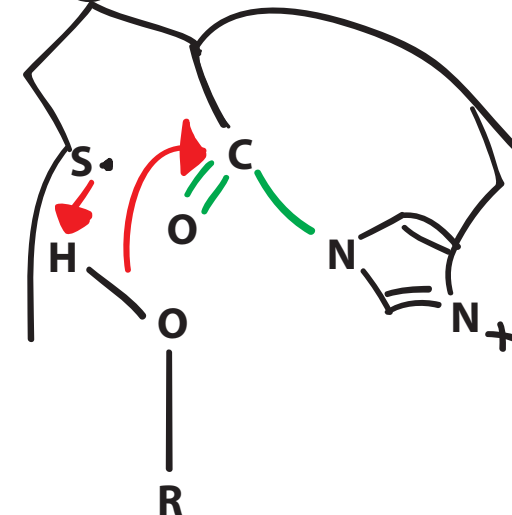
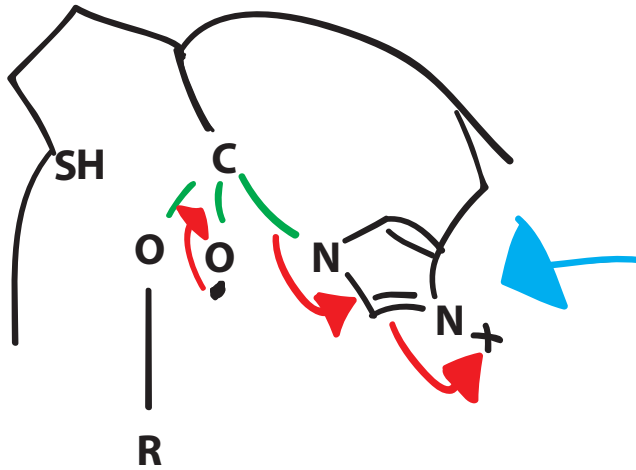
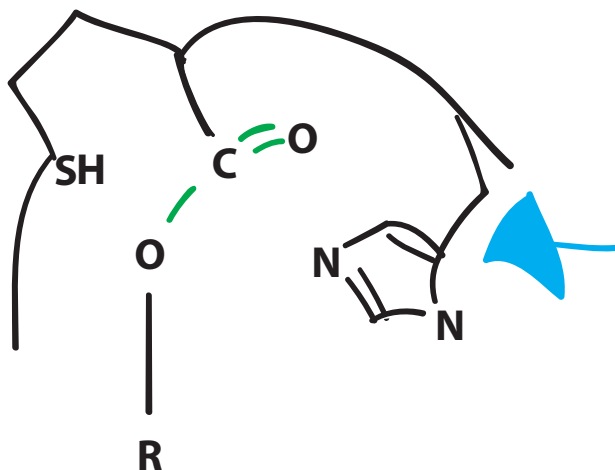
Internal activation



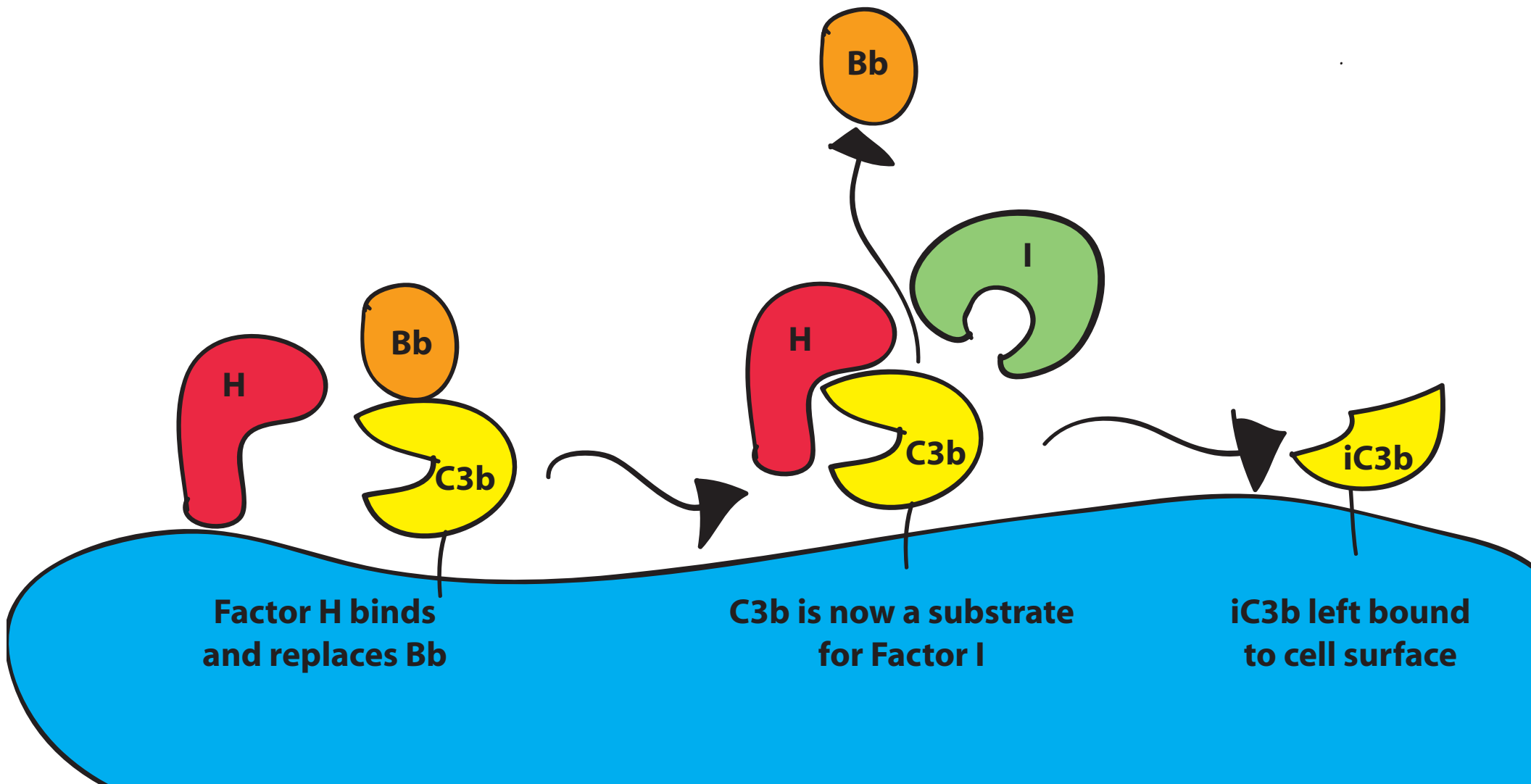
Intermediate



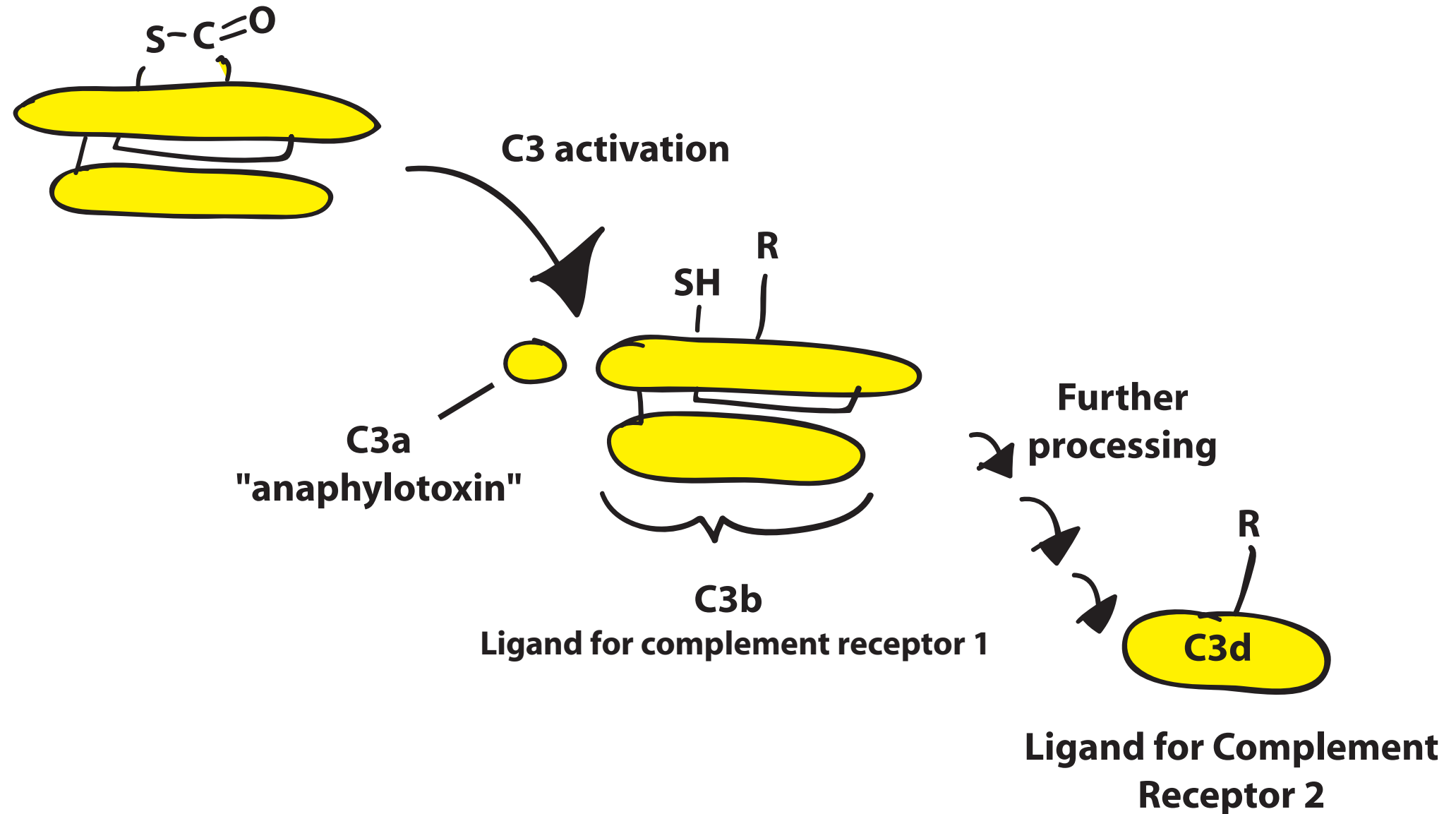
Surface binding



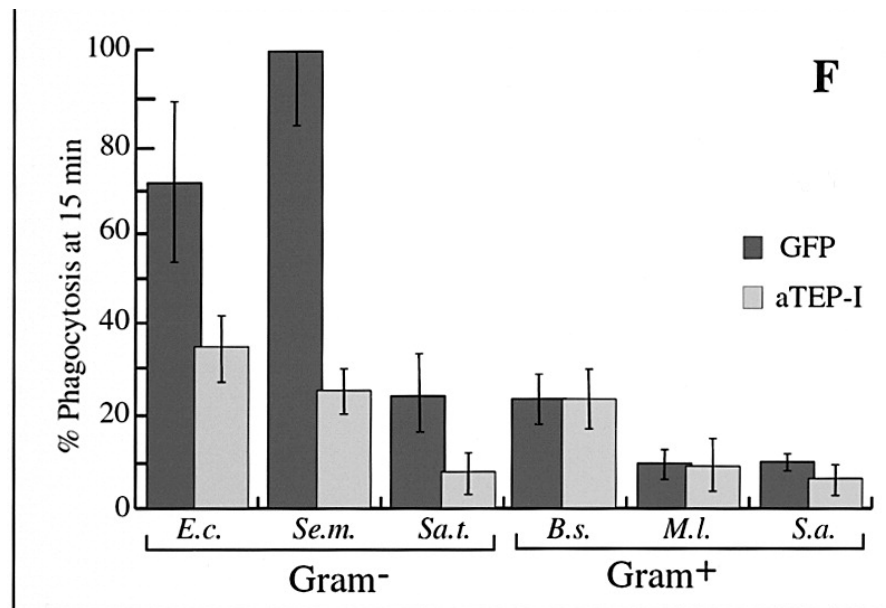
Convertase is deactivated by factors H and I



C3 cleavage produces several active peptides



Inhibition of a thioester protein, aTEP-1, in mosquito cells blocks phagocytosis



Levashina et al. 2001 Cell 104: 709-718