Cross Species Analysis:

Insights Based on Similarities

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September 28, 2011

02- 716: Cross species analysis of genomics data

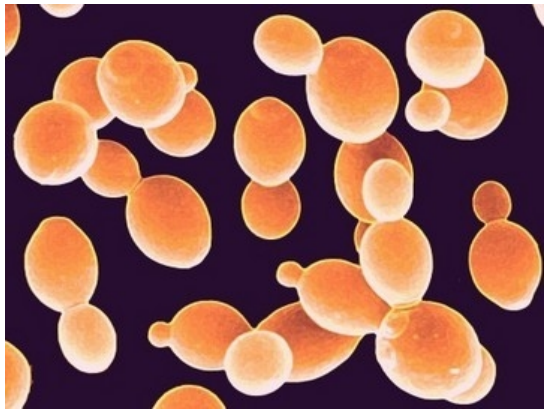
Cross-species comparisons: Overview

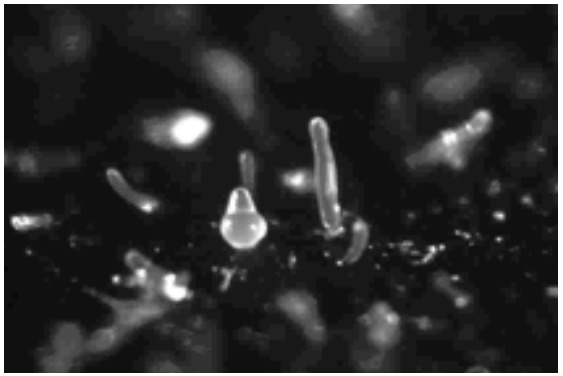
- To review the objective(s) of cross species comparisons.
- To review the use of model organisms.
- To review TLR's as an example of lessons learned from similarities among species.
- To review LRR's as an example of lessons learned from similarities among species.

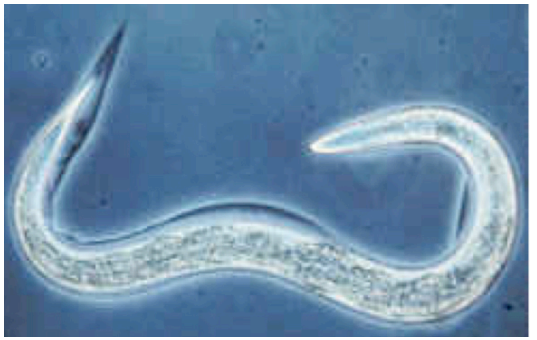
Cross-species comparisons

Why compare?

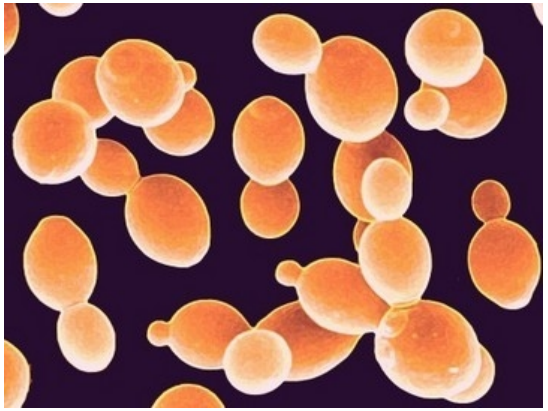
Model Organisms







Model Organisms: Invertebrates



Saccharomyces cerevisiae

Dictyostelium discoideum

Caenorhabditis elegans

Drosophila melanogaster

Model Organisms: Vertebrates

Danio rerio

Mus musculus

Macaca mulatta
(rhesus macaque)

Model Organisms
for Biomedical Research

Mammalian Models:


➤ Mouse


➤ Rat

Non-Mammalian Models:

➤ *S. cerevisiae* (budding yeast)


➤ *S. pombe (fission yeast)*


➤ Neurospora (filamentous fungus)


➤ *D. discoideum* (social amoebae)


➤ *C. elegans* (round worm)


➤ *Daphnia (water flea)*


➤ *D. melanogaster* (fruit fly)


➤ *D. rerio* (zebrafish)


➤ *Xenopus* (frog)


➤ *Gallus (chicken)*

Other Model Organisms:


➤ *Arabidopsis*

Other:

➤ Reports

➤ Funding Opportunities

➤ Process for Considering Support

➤ NIH Policy on Sharing of Model Organisms for Biomedical Research

➤ A User's Guide to the Human Genome

➤ Opportunity to Propose New Organisms for Sequencing

➤ Bacterial Artificial Chromosome (BAC) Resource Network

➤ Rate Setting Manual *for Animal Research Facilities*

➤ Final NIH Statement on Sharing Research Data

➤ Resource Sharing Guidelines

➤ What's New

We hope this web site provides you with information about national and international activities and major resources that are being developed to facilitate biomedical research using the animal models listed here. For organisms not listed, web pages may be developed in the future.

If you have suggestions as to how we can enhance the information provided, please send a message to Bettie Graham at bettie_graham@nih.gov.

Thank you for visiting our web site.

Francis S. Collins, MD, Ph.D.
Director, National Institutes of Health

http://www.nih.gov/science/models/

Model Organisms

- Advantages:
  - ✧ Easy to maintain
  - ✧ Low cost of care
  - ✧ Rapid generation time
  - ✧ Genetic manipulation
  - ✧ Genetic screens

Model Organisms

Model Organisms

- Concerns:
  - ✧ Are discoveries translatable?
  - ✧ Is it necessary to use NHPs?
  - ✧ Difficulty with administrative approvals.
  - ✧ General public's opinion of research.

"The National Anti-Vivisection Society is a national, not-for-profit educational organization incorporated in the State of Illinois. NAVS promotes greater compassion, respect and justice for animals through educational programs based on respected ethical and scientific theory and supported by extensive documentation of the cruelty and waste of vivisection."

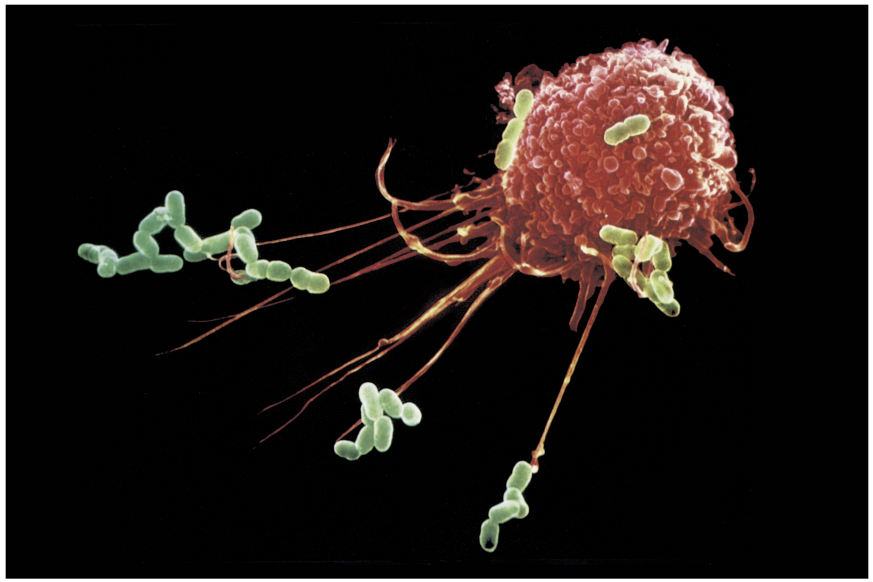
Cross-species comparisons: Innate Immunity

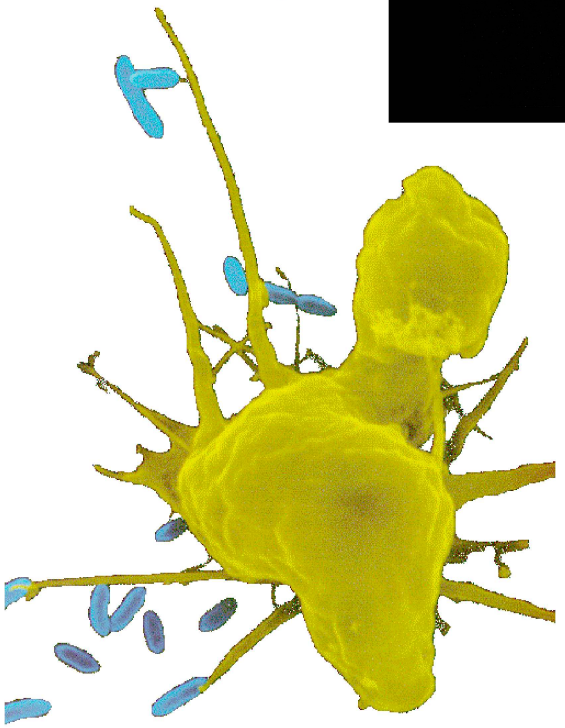
- Biological system of defenses against infections
- Non-cellular components:
  - ✓ coagulation
  - ✓ complement system
  - ✓ antimicrobial peptides
- Cellular components:
  - ✓ macrophages
  - ✓ microphages (neutrophils)

Cross-species comparisons: Innate Immunity - 2

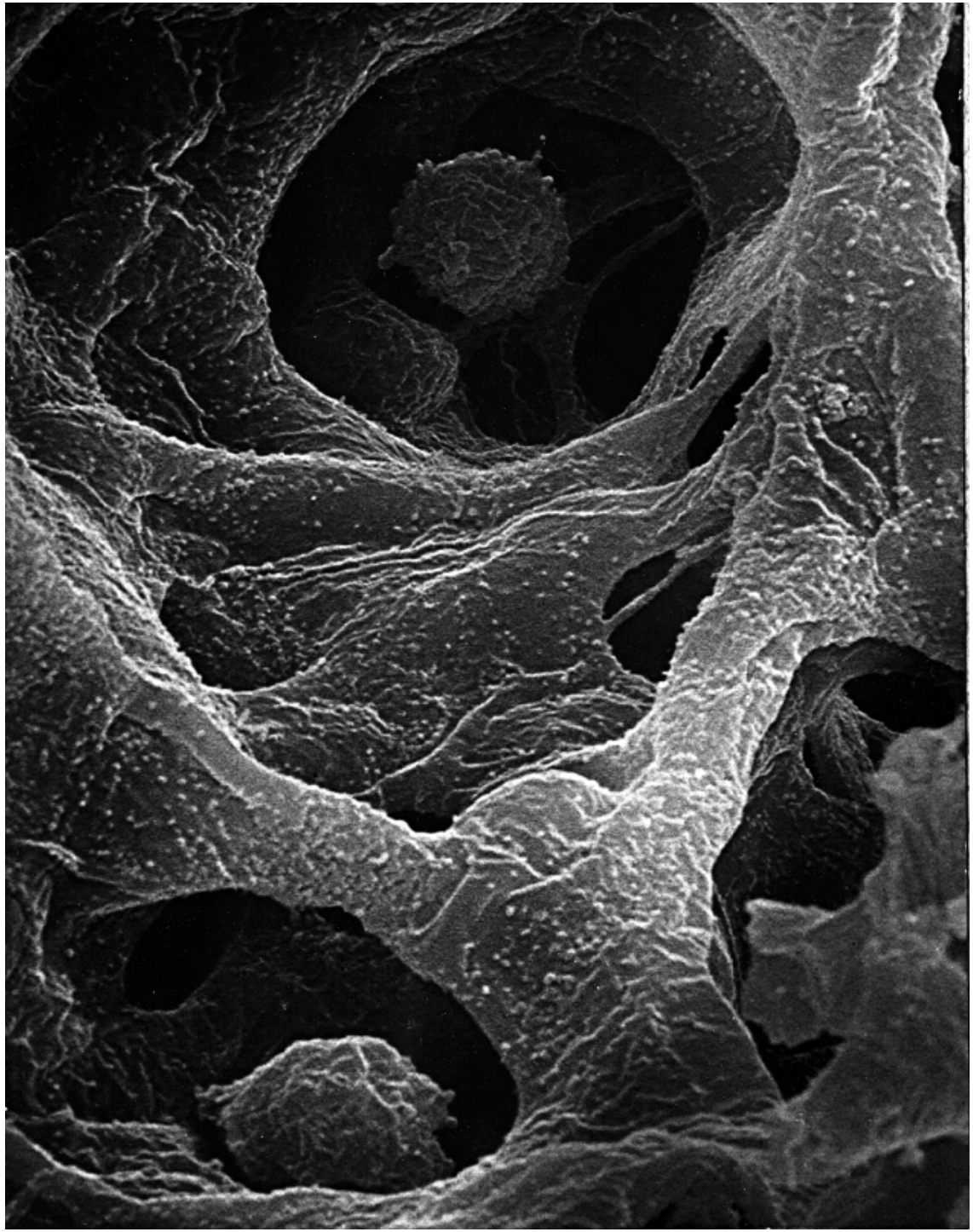
- "Non-specific"
  - ✓ inflammation initiated by many stimuli
- "Adaptive Immunity"
  - ✓ T and B lymphocytes
  - ✓ "antigen receptors" on surface
  - ✓ Specific to particular peptide or carbohydrate

Innate Immunity: Macrophages





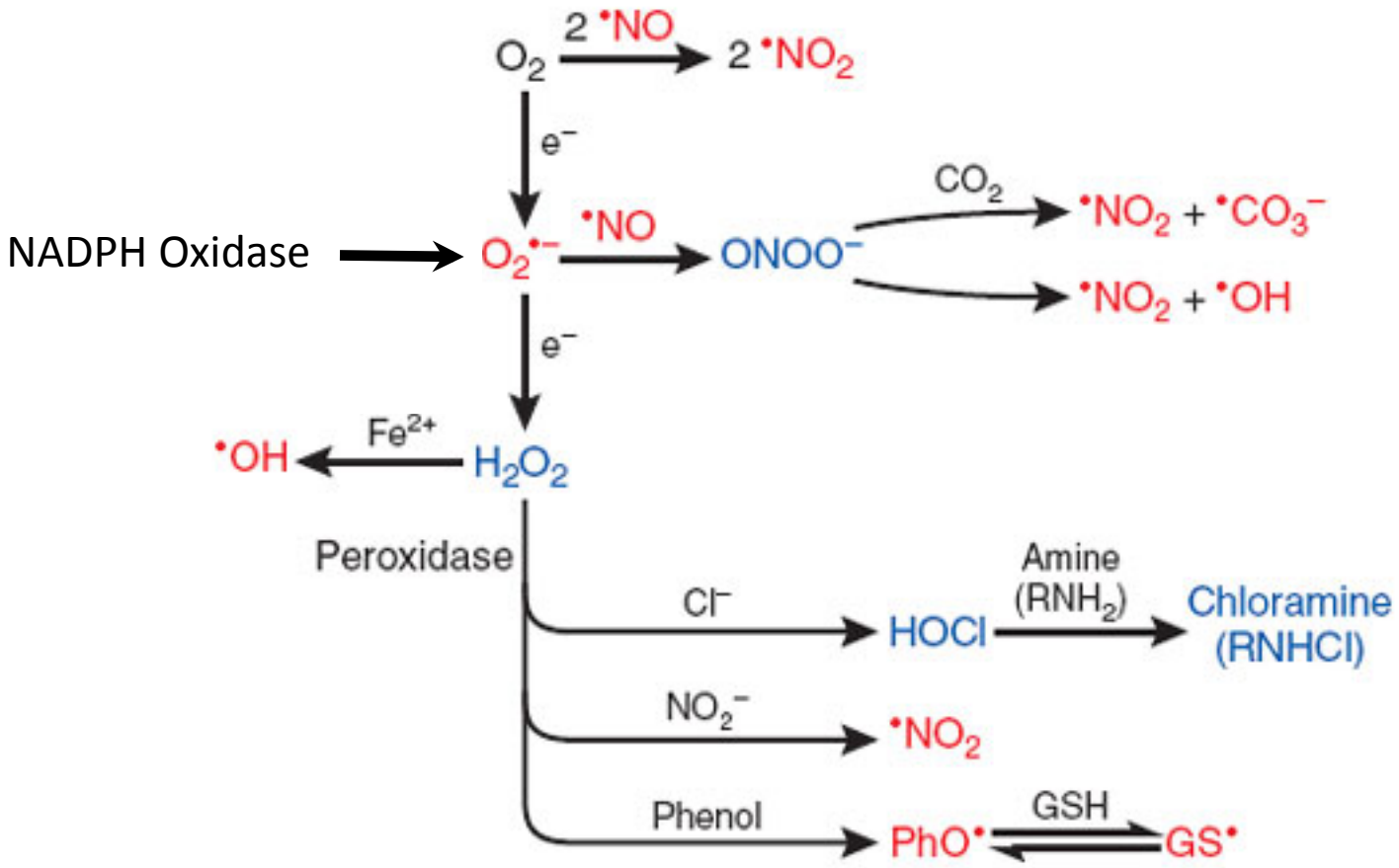
Macrophages in situ: Alveoli and distal airways



Innate Immunity:
Macrophages Antimicrobial Effectors

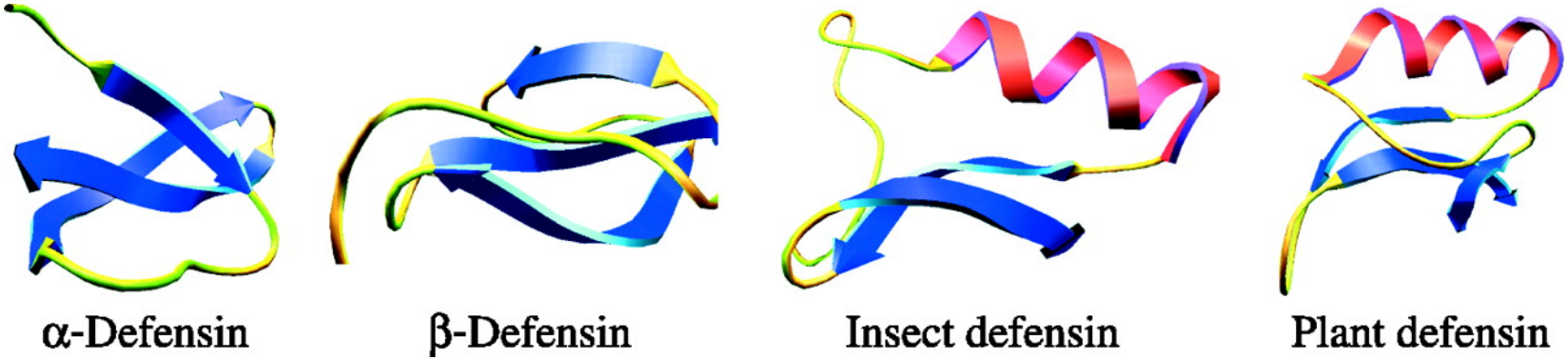
- Phagocytosis
- Reactive oxygen species
- Reactive nitrogen intermediates
- Enzymes
- Acidification
- Antimicrobial peptides

Innate Immunity:
Macrophages Antimicrobial Effectors



Nature Chemical Biology ***4, 278 - 286 (2008)***

Figure 1 Three-dimensional structures of eukaryotic defensins.



J A Hoffmann et al. Science 1999;284:1313-1318

Cross-species comparisons:

Toll-like Receptors

How is the innate immune system activated?

Cell, Vol. 42, 779–789, October 1985, Copyright © 1985 by MIT 0092–8674/85/

Establishment of Dorsal-Ventral Polarity in the Drosophila Embryo: Genetic Studies on the Role of the *Toll* Gene Product

Kathryn V. Anderson,* Gerd Jürgens, and Christiane Nüsslein-Volhard
Friedrich-Miescher-Laboratorium
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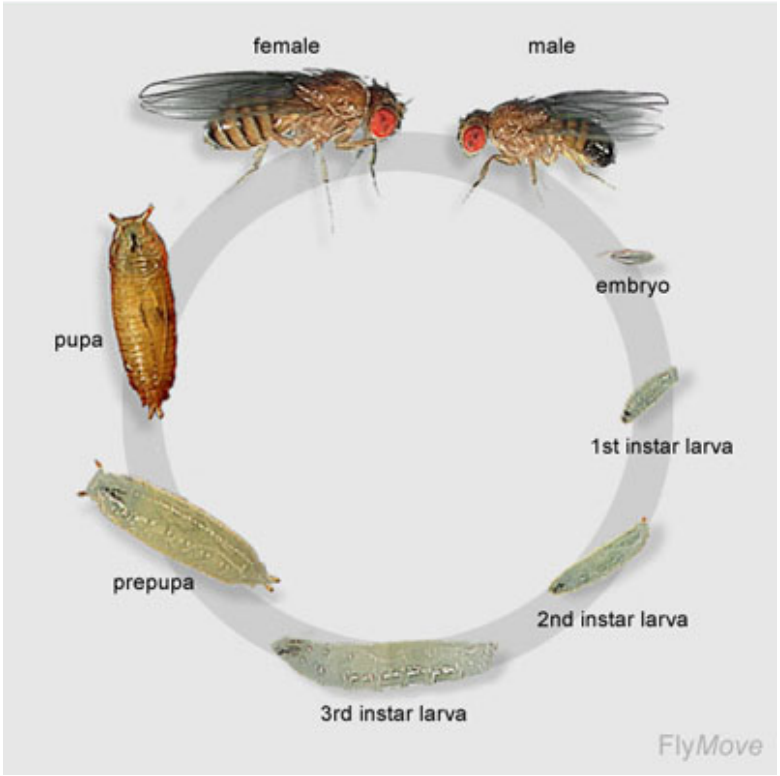


http://www.anatomy.unimelb.edu.au/researchlabs/whitington/index.html

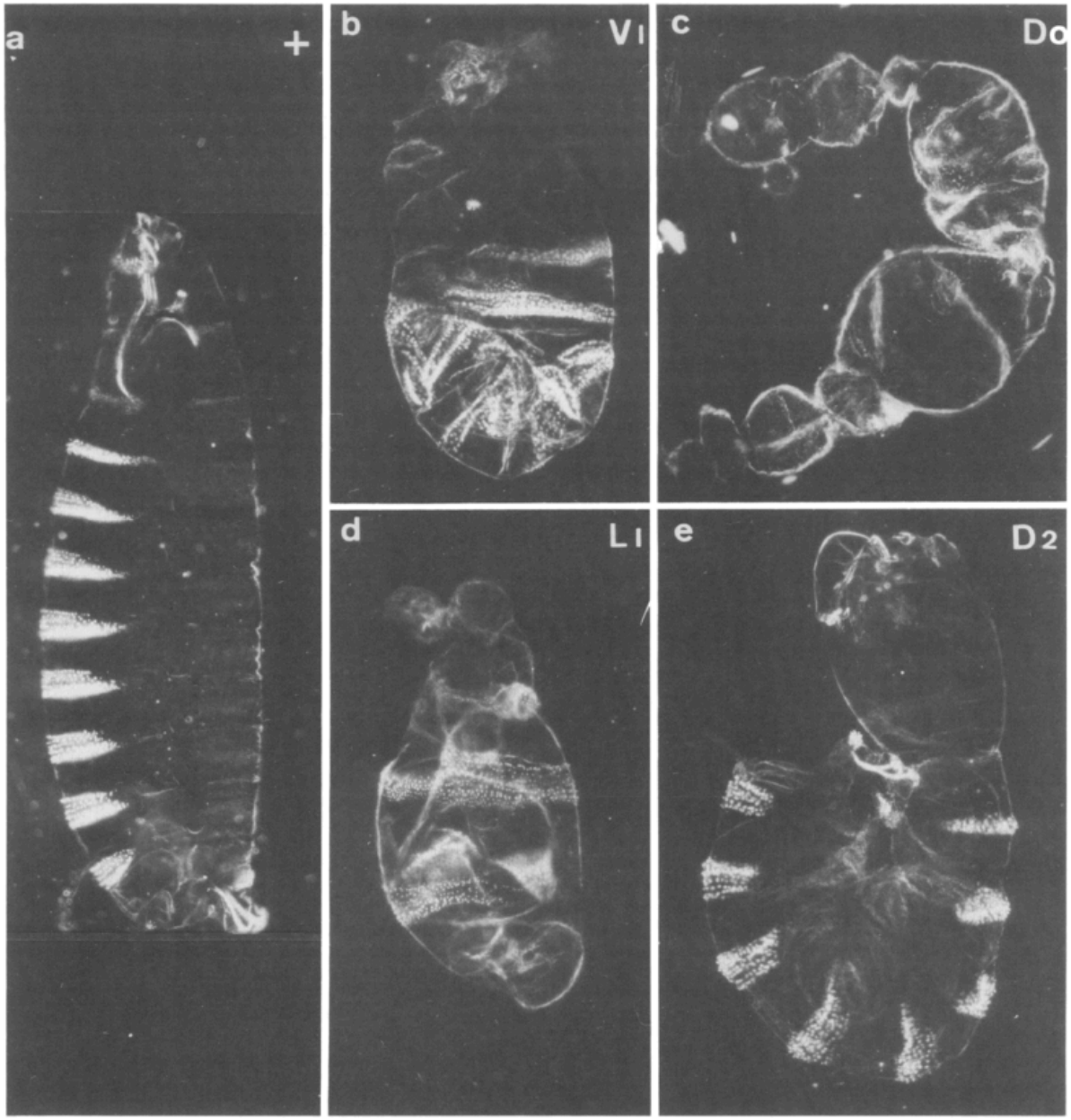


Figure 2. Dark Field Photographs of the Cuticle Produced by *Toll* Mutant Embryos

(a) Wild type (+). (b) Ventralized embryo (V1, according to the nomenclature of Figure 1). Maternal genotype: Tl^{9Q}/+. (c) Dorsalized embryo (D0). Maternal genotype: Tl^{sBREO}/Df(3R)Tl^{9QRX}. (d) Lateralized embryo (L1). Maternal genotype: Tl^{rm10}/Df(3R)ro^{80b}. (e) Partially dorsalized embryo (D2). Maternal genotype: Tl^{r444}/Tl^{r444} (18°C).

Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 9465–9469, October 1995
Genetics

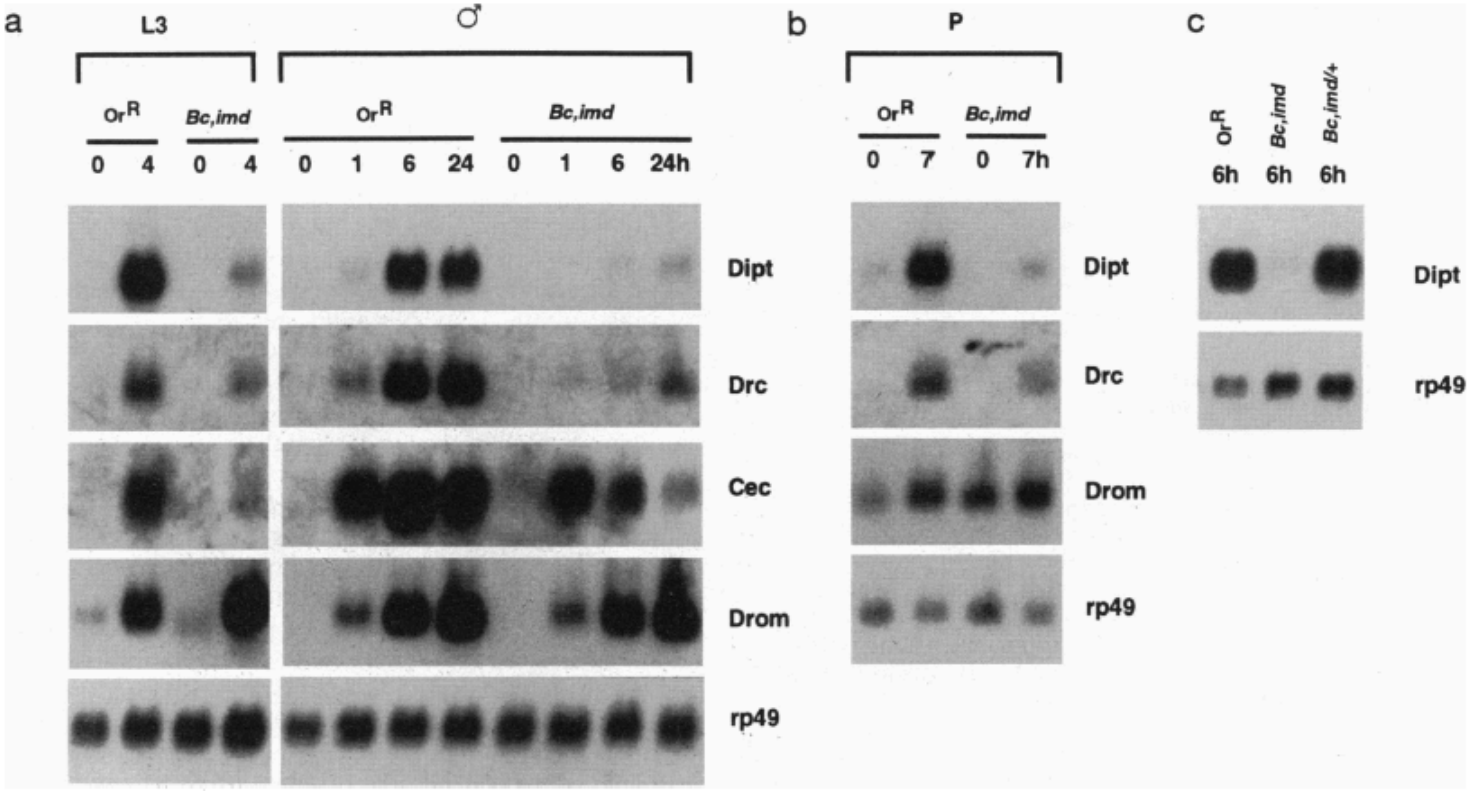
A recessive mutation, immune deficiency (*imd*), defines two distinct control pathways in the *Drosophila* host defense

(antibacterial peptides/antifungal peptides/insect immunity)

BRUNO LEMAITRE*, ELISABETH KROMER-METZGER, LYDIA MICHAUT, EMMANUELLE NICOLAS, MARIE MEISTER, PHILIPPE GEORGEL, JEAN-MARC REICHHART, AND JULES A. HOFFMANN

Institut de Biologie Moléculaire et Cellulaire, Unité Propre de Recherche 9022 du Centre National de la Recherche Scientifique, 15 rue René Descartes–67084, Strasbourg Cedex, France

Communicated by Fotis C. Kafatos, European Molecular Biology Laboratory, Heidelberg, Germany, June 23, 1995 (received for review April 13, 1995)



Cell, Vol. 86, 973–983, September 20, 1996, Copyright ©1996 by Cell Press

The Dorsoventral Regulatory Gene Cassette *spätzle/Toll/cactus* Controls the Potent Antifungal Response in Drosophila Adults

Bruno Lemaitre, Emmanuelle Nicolas, Lydia Michaut, Jean-Marc Reichhart, and Jules A. Hoffmann
Institut de Biologie Moléculaire et Cellulaire
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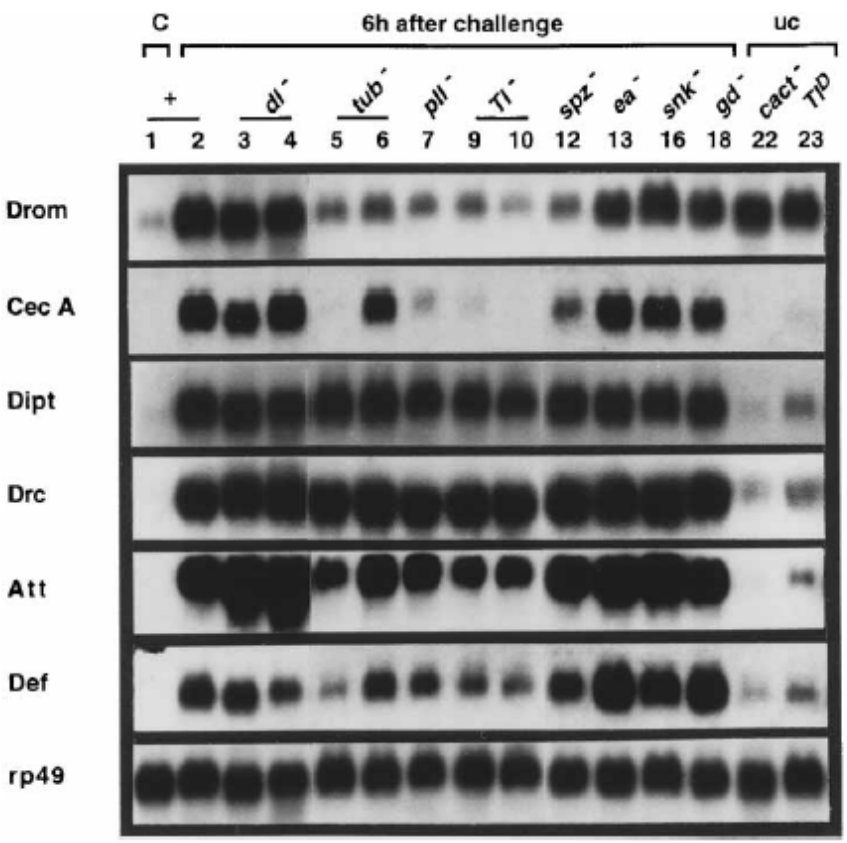
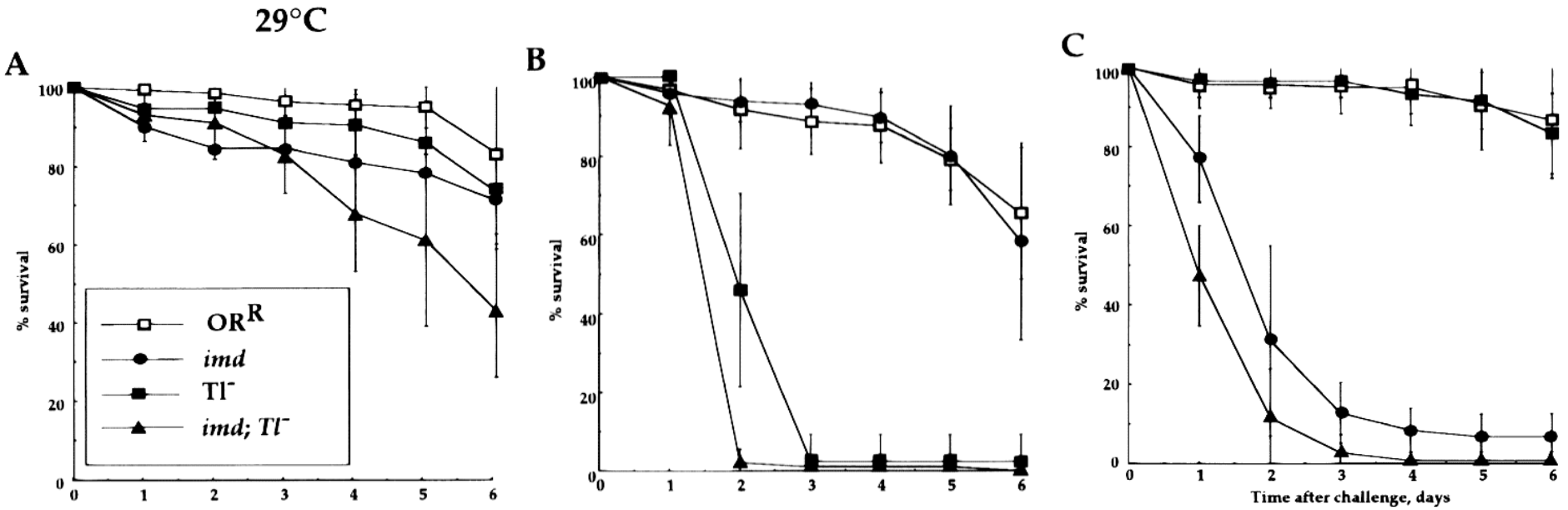
15 rue René Descartes
67084 Strasbourg Cedex
France



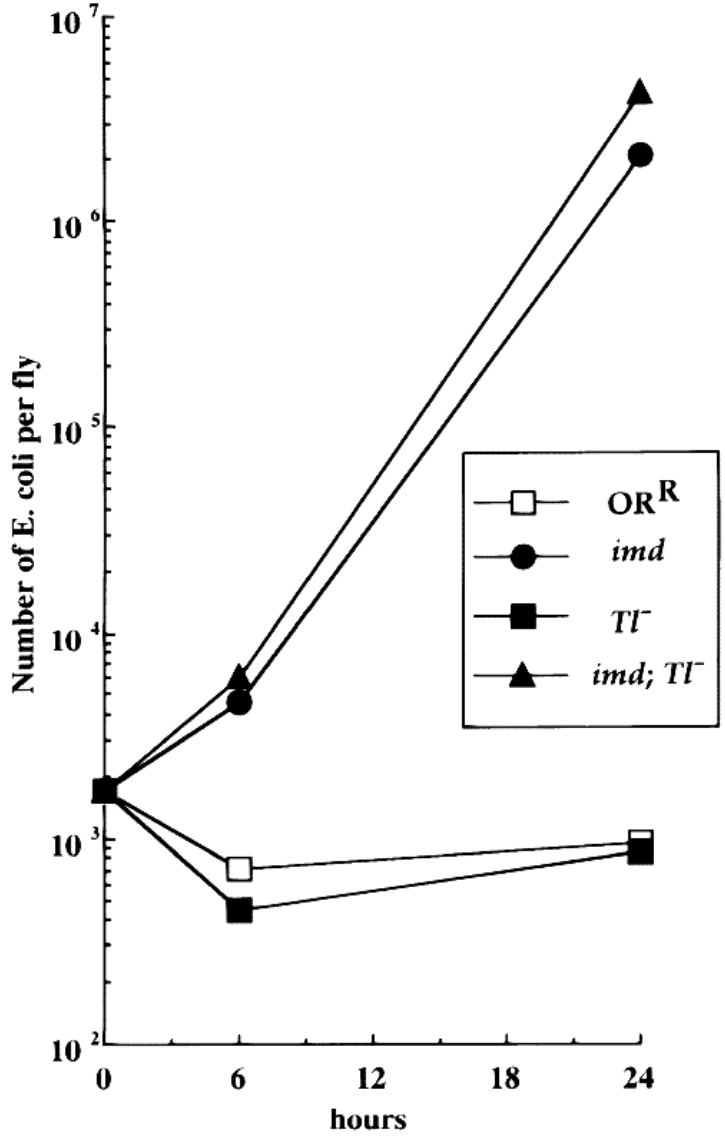
Specificity of Immune Defect



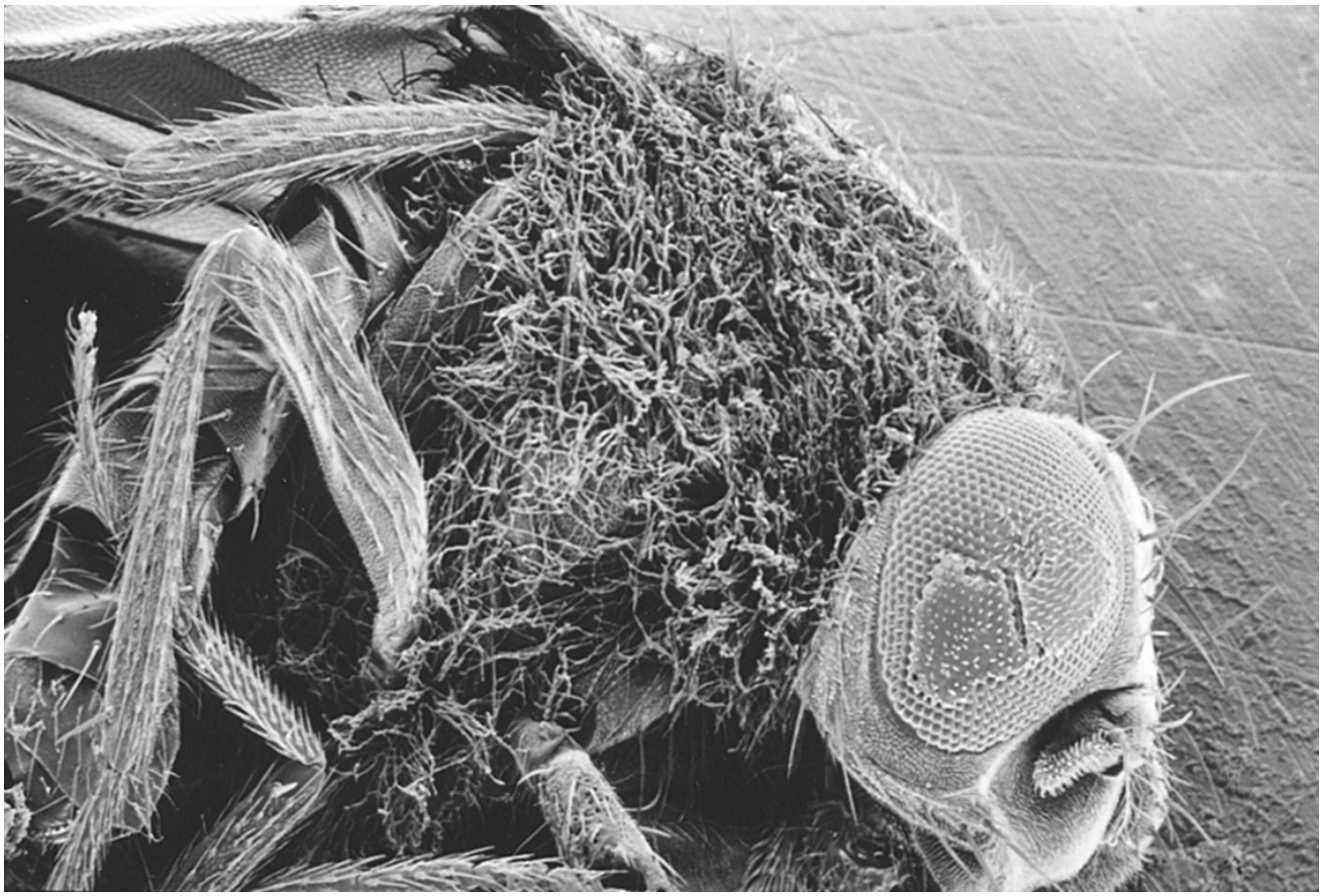
Water | *Aspergillus fumigatus* | *E. coli*

Lemaitre et al. Cell 86:973-983 (1996)

Specificity of Immune Defect



Lemaitre et al. Cell 86:973-983 (1996)



Lemaitre et al. Cell 86:973-983 (1996)

Toll Conclusions and Questions

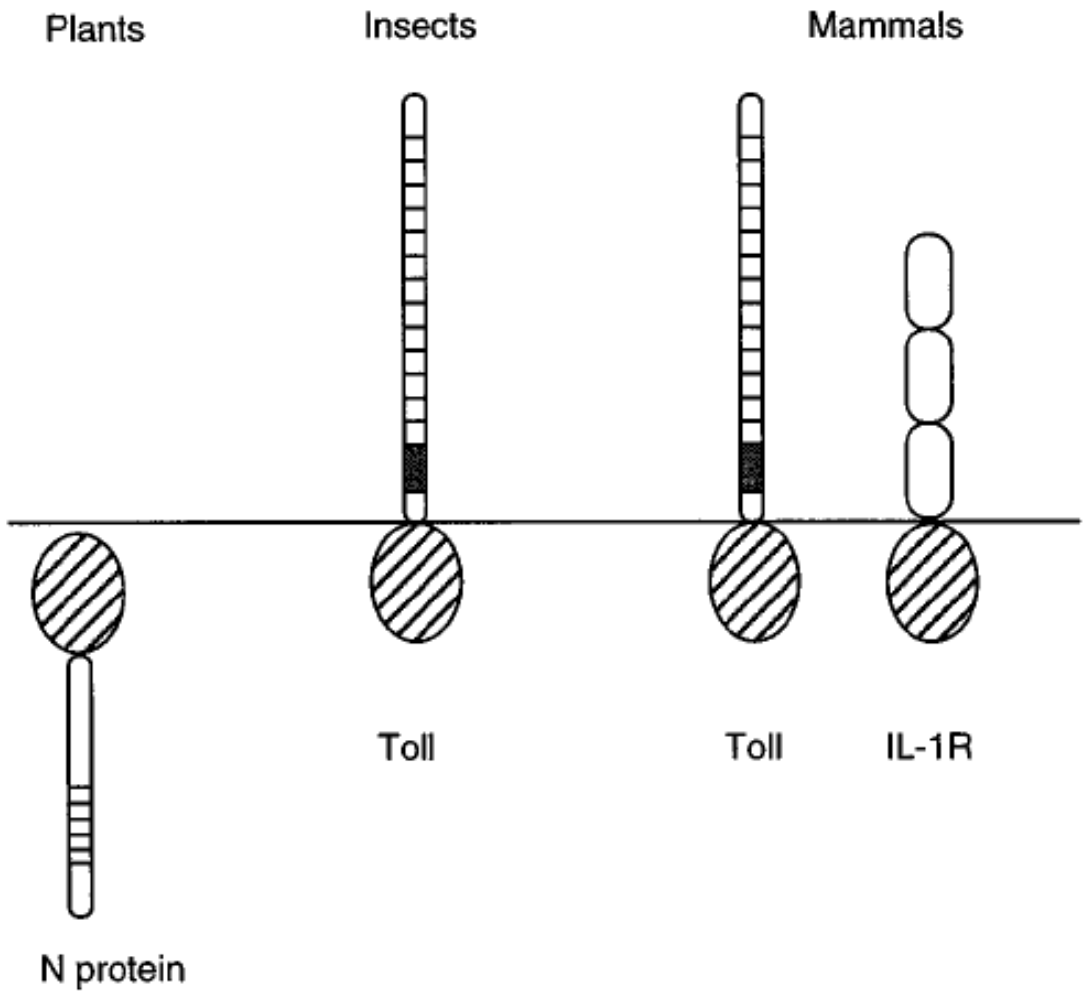
- *Drosophila* have different antimicrobial peptides active against different pathogens.
- Antimicrobial peptide expression is regulated.
- Genetic mutations alter susceptibility to infection.
- Is this finding generalizable?

A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity

Ruslan Medzhitov*, Paula Preston-Hurlburt & Charles A. Janeway Jr*

*Section of Immunobiology, Yale University School of Medicine, and * Howard Hughes Medical Institute, New Haven, Connecticut 06520-8011, USA*

NATURE | VOL 388 | 24 JULY 1997



Medzhitov et al., 1997 *Nature* **388:394-397**

hToll Sequence

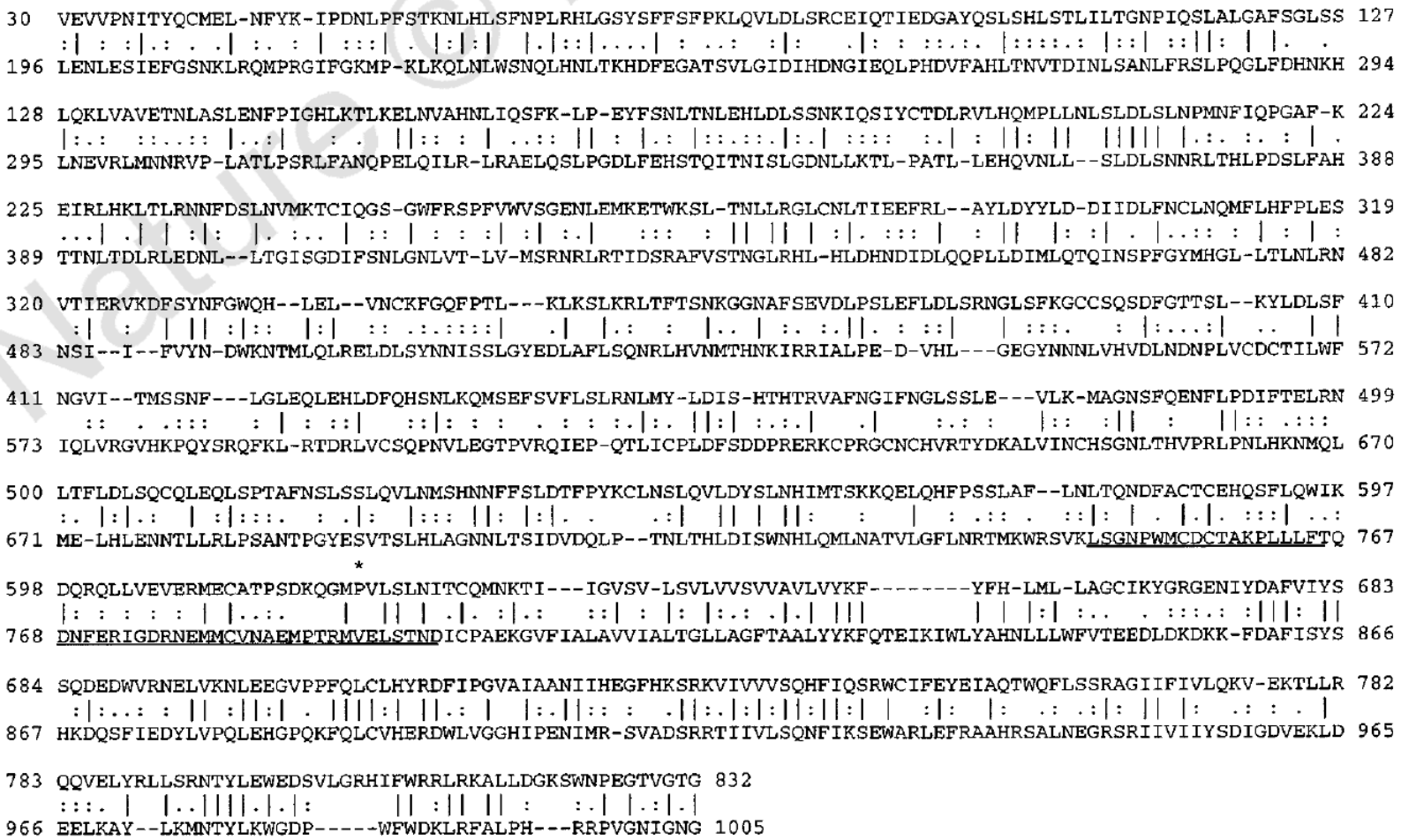
a

```
MISASPLAGT LIPAMAFLSC VKPKSWEPCV EVVPNITYQC MELNFYKIPD NLPFSTKNLH   60
LSFNPLRHLG SYSFFSFPKL QVLDLSRCEI QTIEDGAYQS LSHLSTLILT GNPIQSLALG  120
AFSGLSSLQK LVAVETNLAS LENFPIGHLK TLKELNVAHN LIQSFKLPEY FSNLTNLEHL  180
DLSSNKIQSI YCTDLRVLHQ MPLLNLSLDL SLNPMNFIQP GAFKEIRLHK LTLRNNFDSL  240
NVMKTCIQGS GWFRSPFVWV SGENLEMKET WKSLTNLLRG LCNLTIEEFR LAYLDYYLDD  300
IIDLFNCLNQ MFLHFPLESV TIERVKDFSY NFGWQHLELV NCKFGQFPTL KLKSLKRLTF  360
TSNKGGNAFS EVDLPSLEFL DLSRNGLSFK GCCSQSDFGT TSLKYLDLSF NGVITMSSNF  420
LGLEQLEHLD FQHSNLKQMS EFSVFLSLRN LMYLDISHTH TRVAFNGIFN GLSSLEVLKM  480
AGNSFQENFL PDIFTELRNL TFLDLSQCQL EQLSPTAFNS LSSLQVLNMS HNNFFSLDTF  540
PYKCLNSLQV LDYSLNHIMT SKKQELQHFP SSLAFLNLTQ NDFACTCEHQ SFLQWIKDQR  600
QLLVEVERME CATPSDKQGM PVLSLNITCQ MNKTIIGVSV LSVLVVSVVA VLVYKFYFHL  660
MLLAGCIKYG RGENIYDAFV IYSSQDEDWV RNELVKNLEE GVPPFQLCLH YRDFIPGVAI  720
AANIIHEGFH KSRKVIVVVS QHFIQSRWCI FEYEIAQTWQ FLSSRAGIIF IVLQKVEKTL  780
LRQQVELYRL LSRNTYLEWE DSVLGRHIFW RRLRKALLDG KSWNPEGTVG TGCNWQEATSI 841
```

Medzhitov et al., 1997 *Nature* **388:394-397**

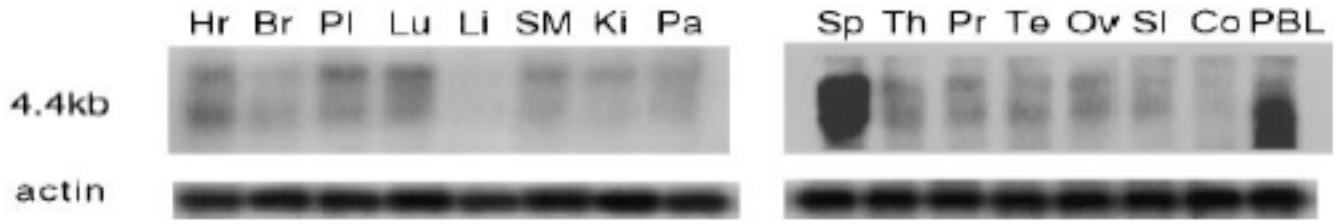
Sequence Comparison hToll and dToll

b

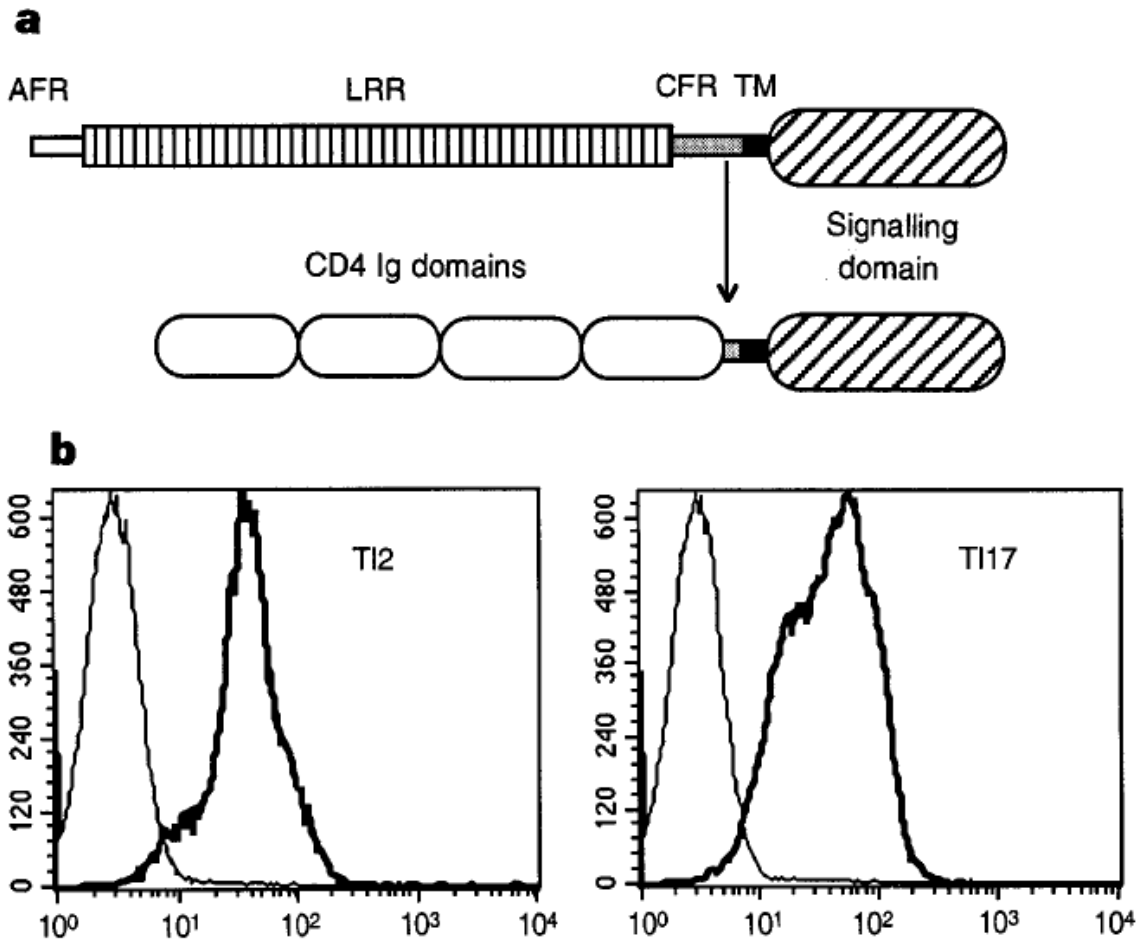


Medzhitov et al., 1997 *Nature* **388:394-397**

hToll Gene Expression

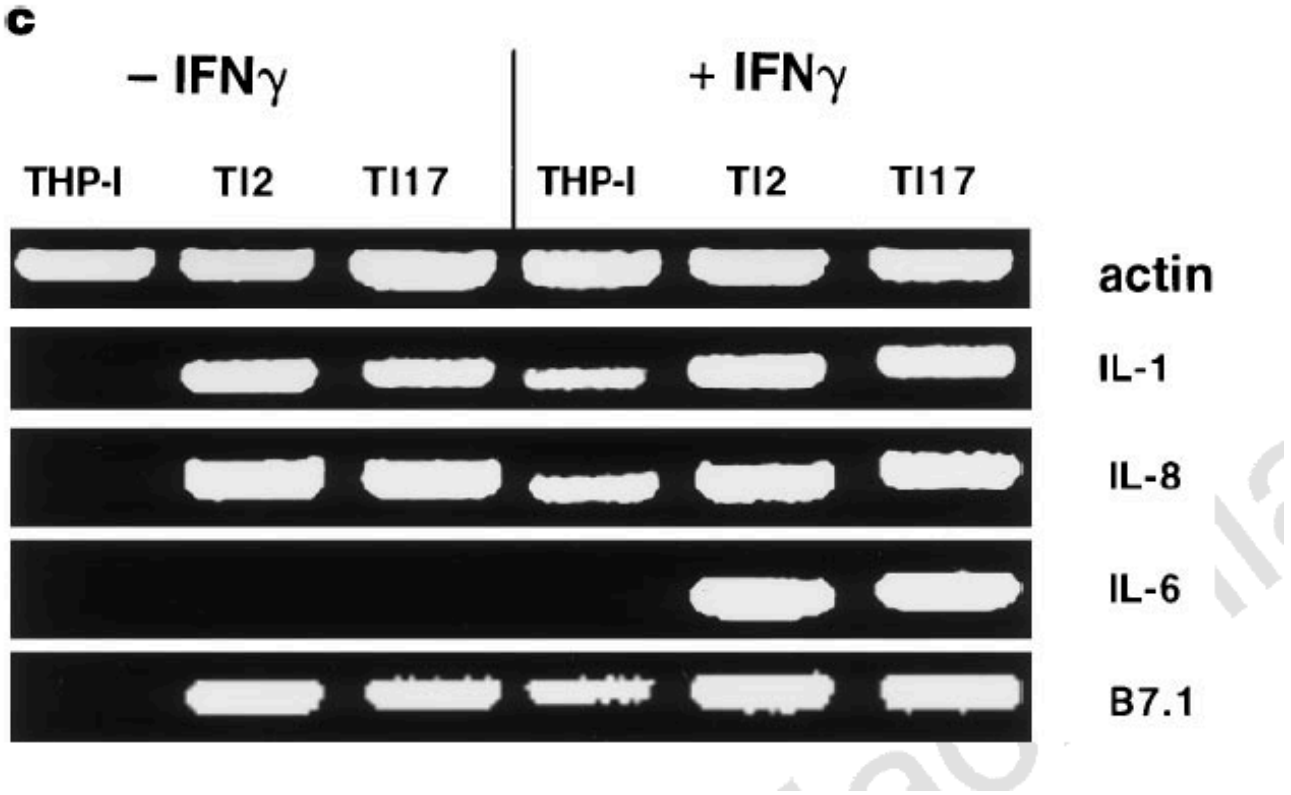


hToll Function



Medzhitov et al., 1997 *Nature* **388:394-397**

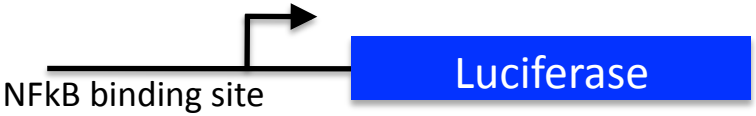
hToll Function

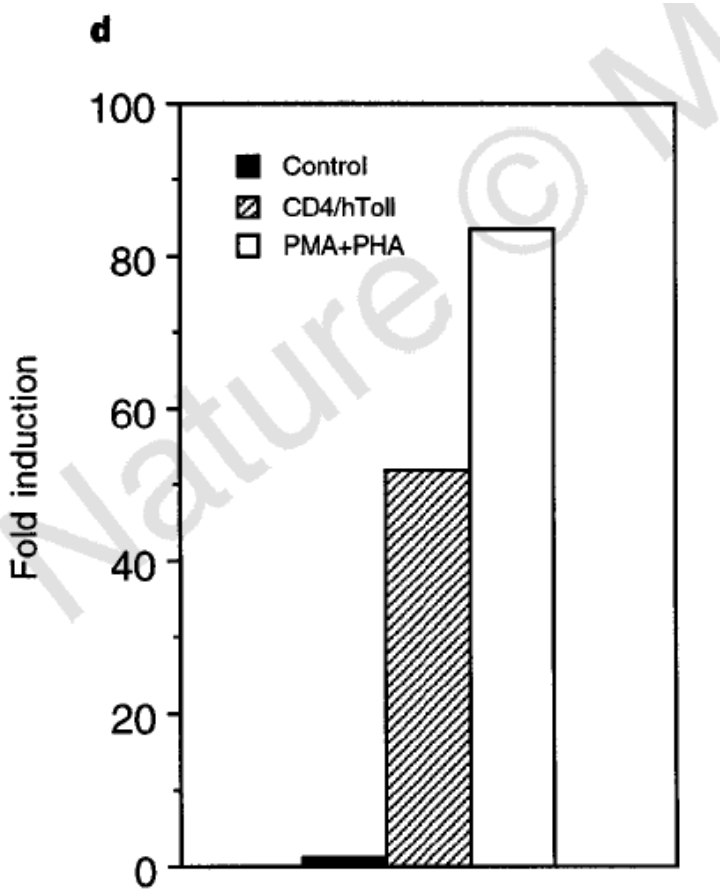


Medzhitov et al., 1997 *Nature* **388:394-397**

hToll Function

Reporter construct:





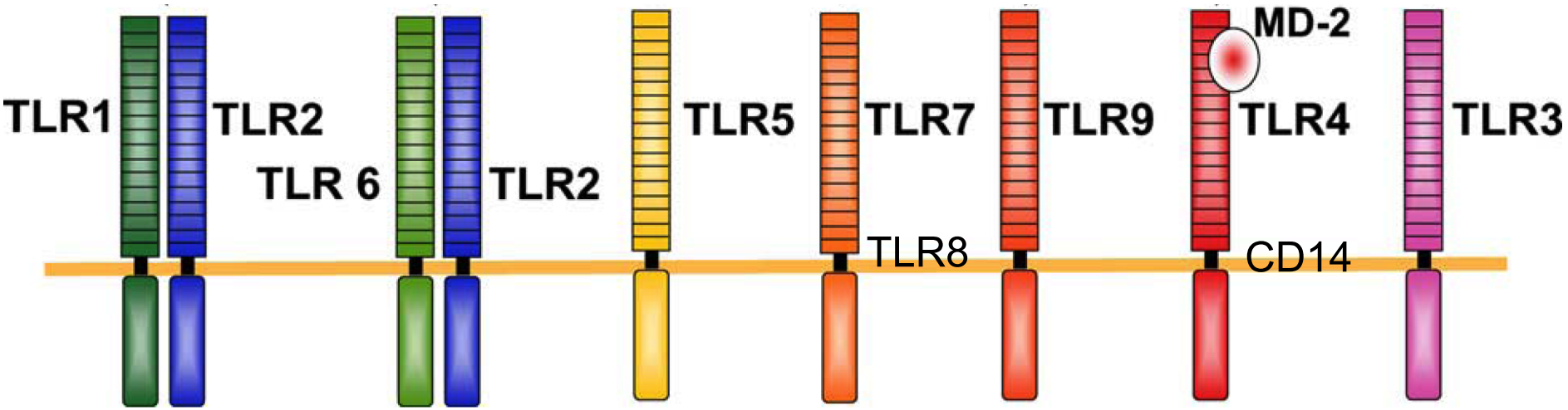
Medzhitov et al., 1997 *Nature* **388:394-397**

hToll Conclusions and Questions

- The exchange of information on innate immune studies set the stage for discovery of dToll.
- dToll has a fortuitous double use in development and innate immunity.
- A simple homology search led to the discovery of hToll.
- What molecule(s) is(are) recognized by hToll?
- Is there specificity in the human innate immune response?

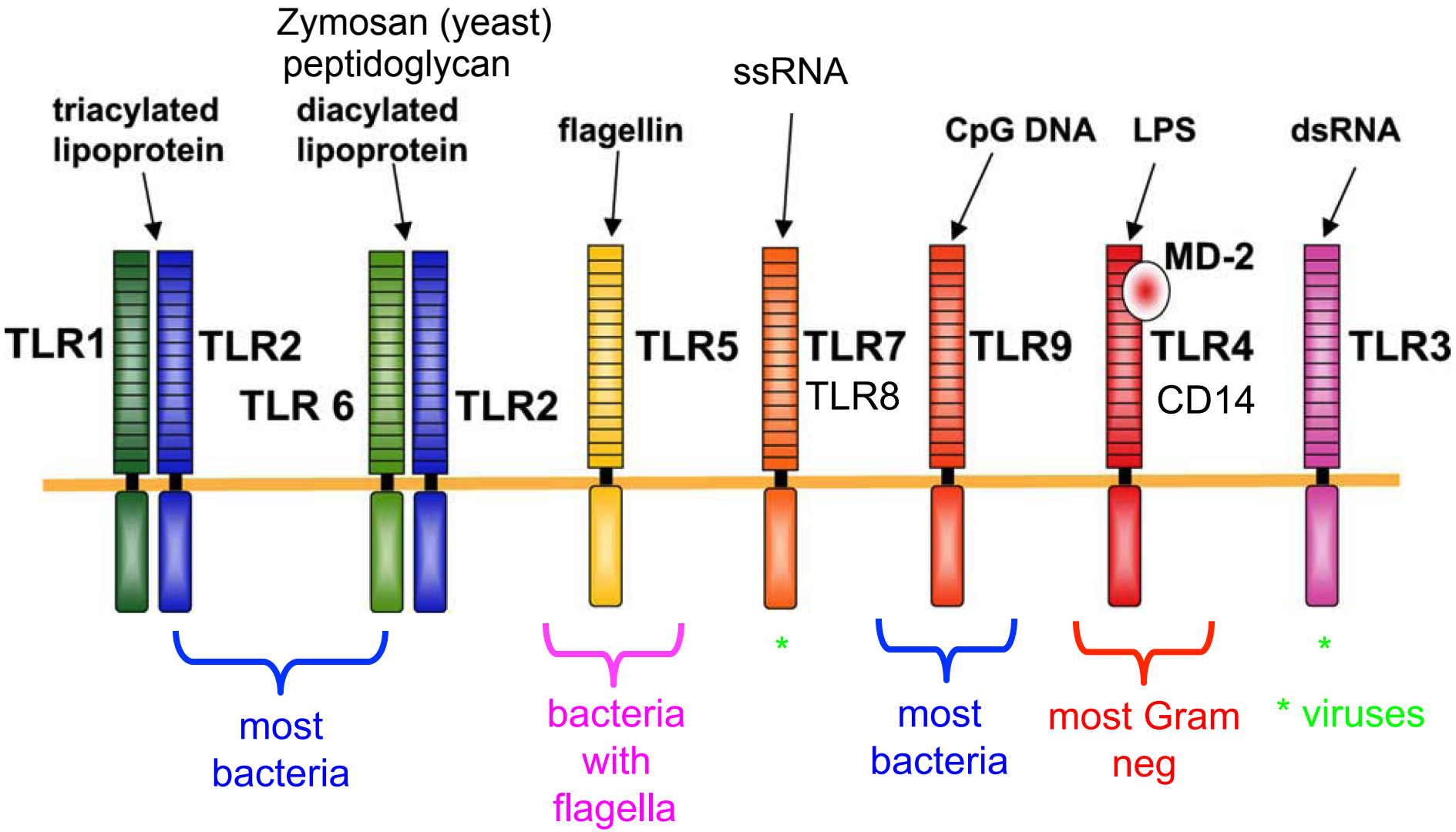
Initiation of Inflammation: Toll-like Receptors

monocytes, macrophages, dendritic cells, neutrophils, endothelial cells



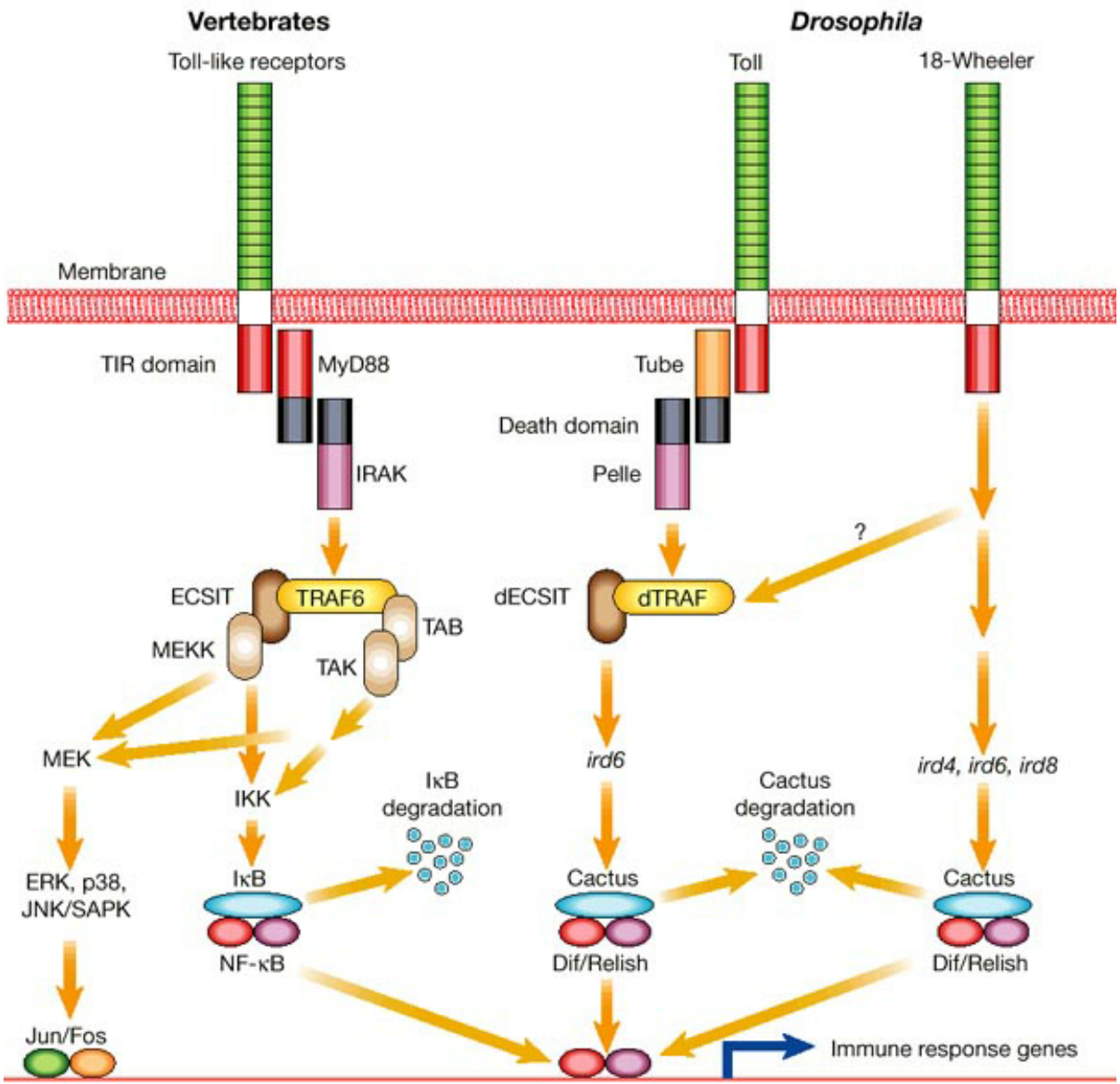
Seminars in Immunology 16 (2004) 3–9

TLR “Ligands”



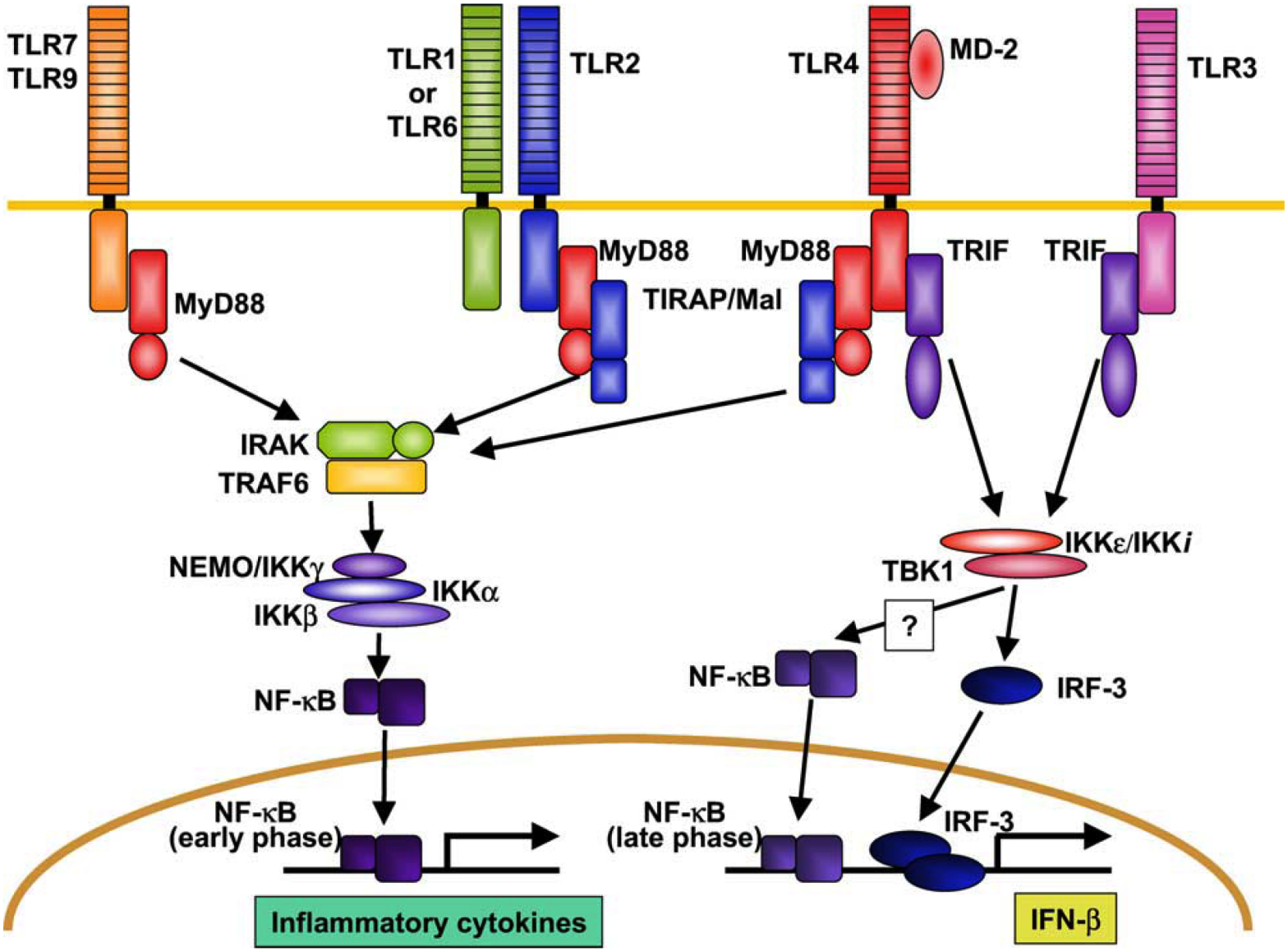
Seminars in Immunology 16 (2004) 3–9

Toll In Drosophila and Vertebrates



Aderem and Ulevitch Nature 406, 782 - 787 (2000)

TLR Signaling



Seminars in Immunology 16 (2004) 3–9

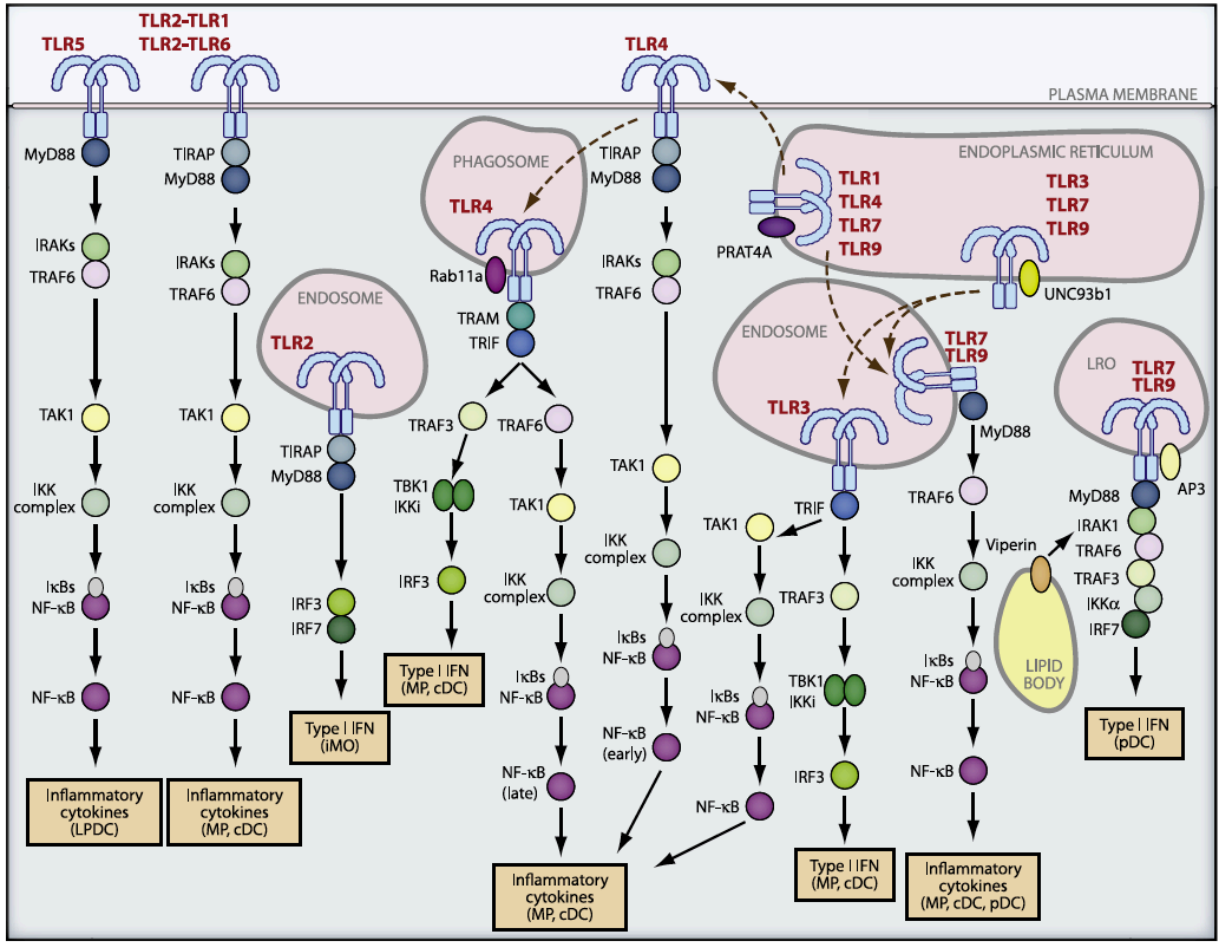


Figure 1. TLR Trafficking and Signaling

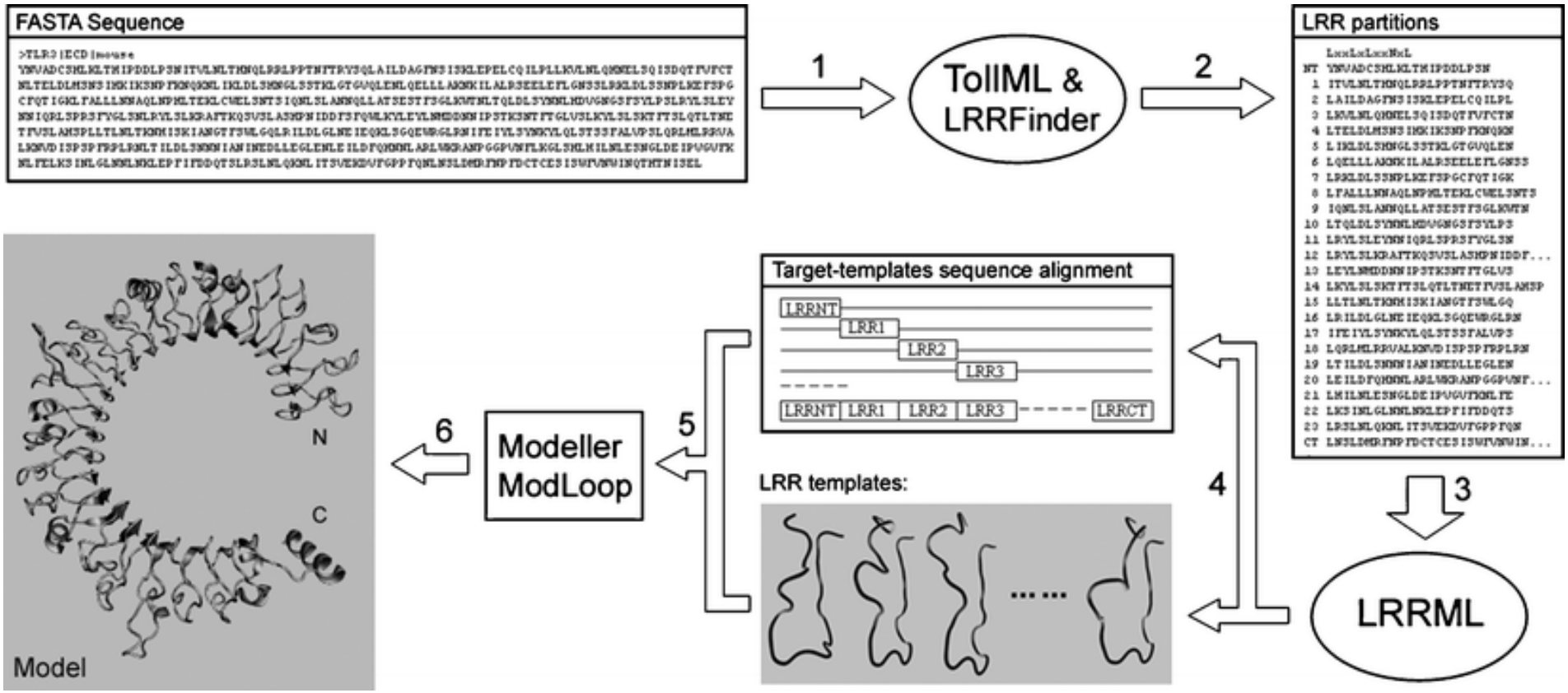
Individual TLRs initiate overlapping and distinct signaling pathways in various cell types such as macrophages (MP), conventinal DC (cDC), plasmacytoid DC (pDC), lamina propria DC (LPDC), and inflammatory monocytes (iMO). PAMP engagement induces conformational changes of TLRs that allow homo- or heterophilic interactions of TLRs and recruitment of adaptor proteins such as MyD88, TIRAP, TRIF, and TRAM. TLR5, which is highly expressed on the cell surface of LPDC, uses MyD88 and activates NF-κB through IRAKs, TRAF6, TAK1, and IKK complex, resulting in induction of inflammatory cytokines. Heterodimers of TLR1-TLR2 and TLR2-TLR6 are also expressed on the cell surface and induce NF-κB activation through recruitment of TIRAP and MyD88 in macrophages and cDCs. In iMO, TLR2 is found to be expressed within the endosome and induce type I IFN via IRF3 and IRF7 in response to viruses. TLR4, which is expressed on the cell surface, initially transmits signals for the early-phase activation of NF-κB by recruiting TIRAP and MyD88. TLR4 is then transported into Rab11a-positive phagosomes that contain bacteria, where it recruits TRAM and TRIF and activates TRAF3-TBK1-IRF3 axis as well as late-phase NF-κB activation for the induction of type I IFN. Both early- and late-phase activation of NF-κB is required for the induction of inflammatory cytokines. TLR3, TLR7, and TLR9 are localized mainly to the ER in the steady state and traffic to the endosomal compartment, where they engage with their ligands. UNC93B1, which interacts with these TLRs in the ER, mediates this trafficking. The translocation of TLR7 and TLR9 from the ER to the endosome is also regulated by PRAT4A, which also supports the translocation of TLR4 and TLR1 to the cell surface. A member of ER-resident gp96 functions as a general chaperone for most TLRs including TLR1, TLR2, TLR4, TLR5, TLR7, and TLR9 (not shown here). TLR3 activates the TRIF-dependent pathway to induce type I IFN and inflammatory cytokines in macrophages and cDCs. In pDCs, TLR7 and TLR9 activate NF-κB and IRF7 via MyD88 to induce inflammatory cytokines and type I interferon, respectively. The activation of NF-κB during TLR7 and TLR9 signaling is initiated from the endosome whereas IRF7 activation is initiated from the lysosome-related organelle (LRO) after TLR7 and TLR9 are transported from the endosome to this vesicle in a manner dependent on AP3. MyD88-dependent IRF7 activation in pDCs is mediated by activation of IRAK1, TRAF6, TRAF3, and IKKα and is facilitated by IFN-inducible Viperin expressed in the lipid body. In cDCs and macrophages, TLR7 and TLR9 induce inflammatory responses by activating NF-κB via MyD88 but fail to activate IRF7.

Wiki Commons

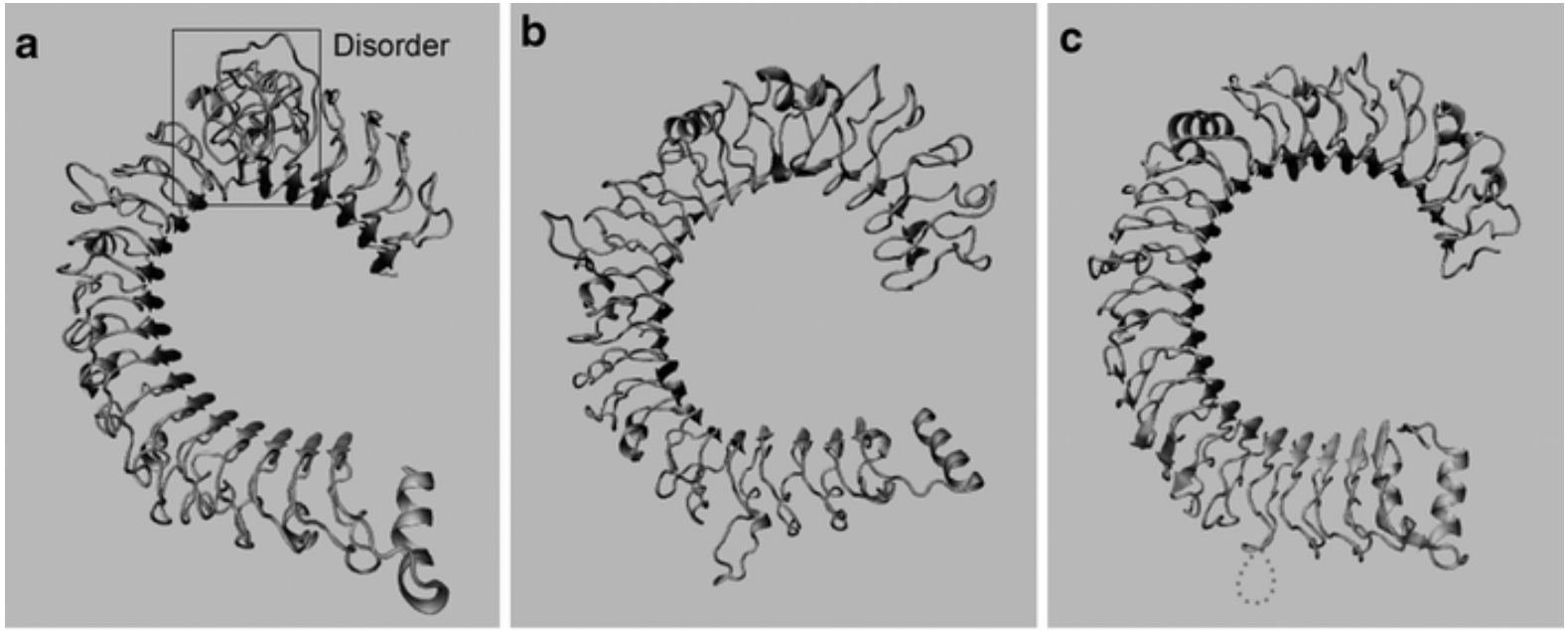
Extension of Structural Analyses of TLR: Analysis of Leucine Rich Repeats

- Most LRR domains consist of a chain of between 2 and 45 LRRs.
- Each repeat in turn is typically 20 to 30 residues long.
- can be divided into a highly conserved segment (HCS) followed by a variable segment (VS).
- The HCS usually consists of either the 11-residue sequence LxxLxLxxNxL or the 12-residue sequence LxxLxLxxCxxL, where L is Leu, Ile, Val, or Phe; N is Asn, Thr, Ser, or Cys; and C is Cys, Ser, or Asn.

Ng A C Y et al. *PNAS* 2011;108:4631-4638



Wei et al., *J Molecular Modeling 2011,* ***17(1):27-36***

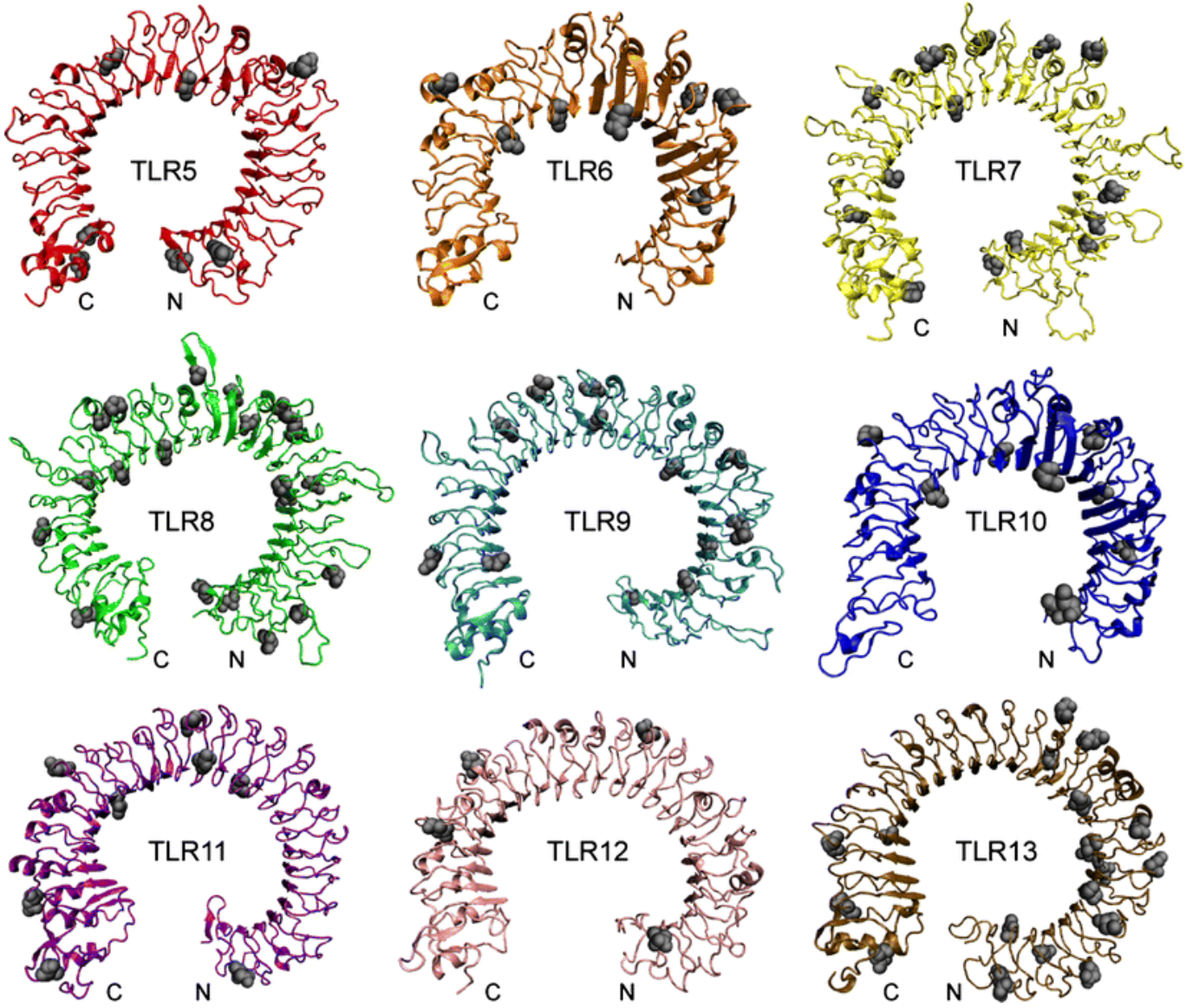


d

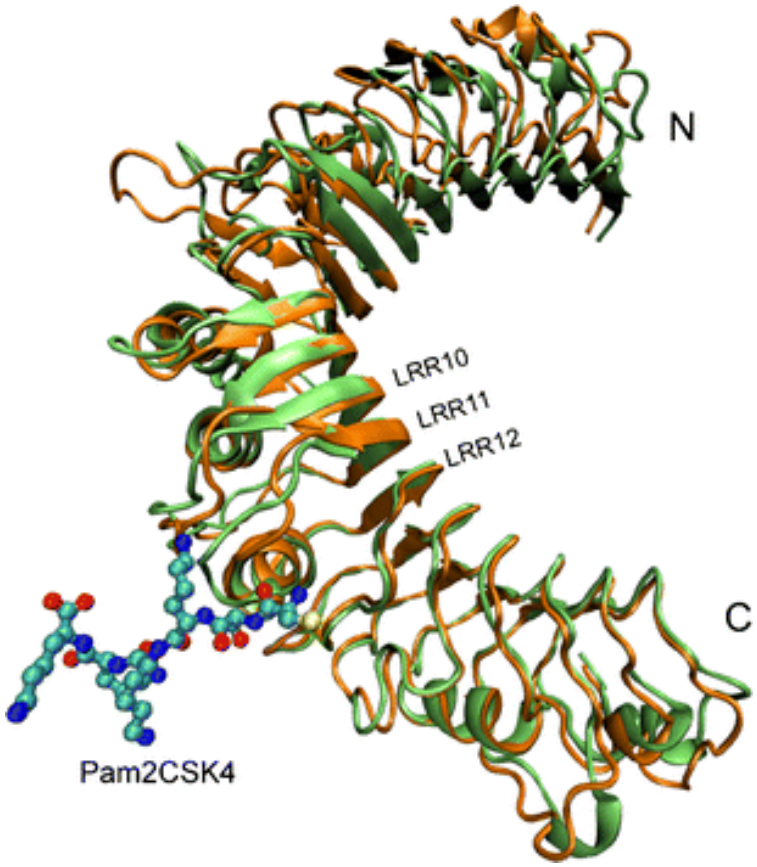
```
                  LRR6                                LRR7                                LRR8
mTLR3 |LQELLLAKNKILALRSEELEFLGNSS|LRKLDLSSNPLKEF-SPGCFQTIGK|LFALLLNNAQLNPHLTEKLCWELSNTS|
2Z64  |LVHVDLSYNYIQTITVNDLQF-----|-----LRENPQVNL-----------|--SLDMSLNPIDFIQ---------DQA|
2Z81  |--------------------------|--------------------------|---------PIDFIQ---------DQA|
1O6V  |--------------------------|--------------------GSATI|TQDTPINQIFTDTALAEKMKTVLGKTN|
3FXI  |LQKLVAVETNLASLEN--FPIGHLKT|LKELNVAHNLIQSFKLPEYFSNLTN|LEHLDLSSNKIQSIYCTDLRVLHQM--|
2Z7X  |--------------------------|--------------------------|---------------------------|
1JL5  |--------------------------|-----KSKTEYYNA-WSEWERNA--|-----PPGNGEQREMAVSRLRDCLDRQ|
3BZ5  |--------------------------|--------------------TLKA|GQTQSFNDWFPDDNFASEVAAAFEMQA|

                  LRR9                                LRR10
mTLR3 |IQ-------NLSLANNQLLATSESTFSGLK---------WTN|LTQLD---LSYNNLHDV-GNGSFSYLPS|
2Z64  |FQGIKLHELTLRGNFNSSNIMKTCLQNLAGLHVHRLILGEFK|---------DERNLEIF-EPSIMEGLCD|
2Z81  |TF-------SEIRRI-----------DFAG---------LTS|LNELEIKALSLRNYQS-------QSLKS|
1O6V  |VT-------DTVS--------------QTD---------LDQ|VTTLQ---ADRLGIKSI-D--GVEYLNN|
3FXI  |-------------------------------------PLL|NLSLD---LSLNPMNFI-QP---GAFKE|
2Z7X  |---------KISCH--------------P---------TVN|LKHLD---LSFNAFDALPICKEFGNMSQ|
1JL5  |AH-------ELELNNLGLSSLP------EL---------PPH|LESLV---ASCNSLTEL-P----ELPQS|
3BZ5  |TD-------TISEEQLA-----------------------T|LTSLD---CHNSSITDM-TG--IEKLTG|
```

Wei et al., *J Molecular Modeling 2011,* ***17(1):27-36***



Wei et al., *J Molecular Modeling 2011,* ***17(1):27-36***



Wei et al., *J Molecular Modeling 2011,* ***17(1):27-36***

Human leucine-rich repeat proteins: a genome-wide bioinformatic categorization and functional analysis in innate immunity

Aylwin C. Y. Ng[a,b,1], Jason M. Eisenberg[a,b,1], Robert J. W. Heath[a], Alan Huett[a], Cory M. Robinson[c], Gerard J. Nau[c], and Ramnik J. Xavier[a,b,2]

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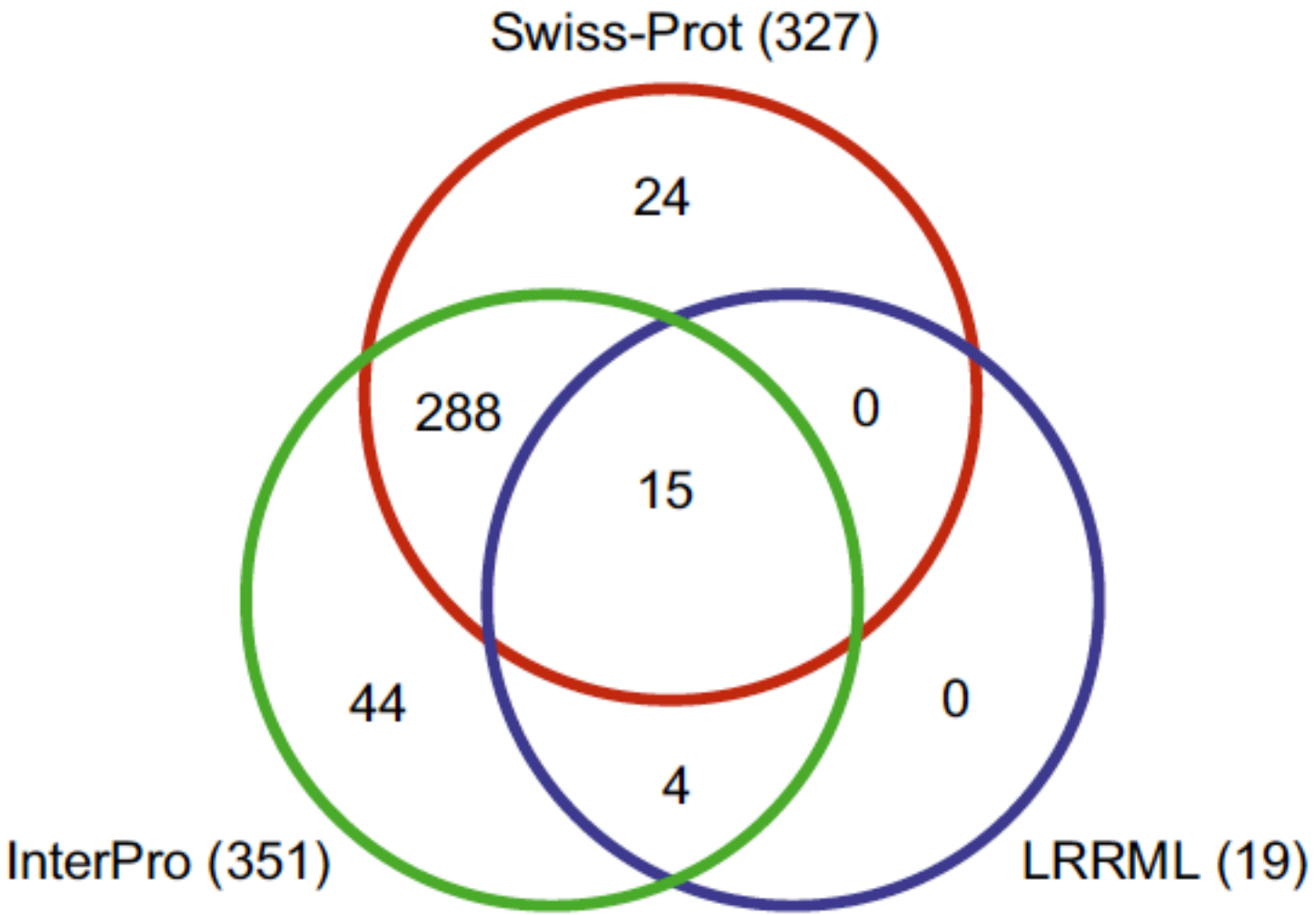
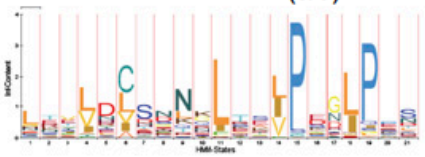
LRR Proteins in Public Databases



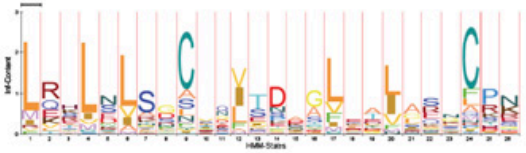
Ng A C Y et al. *PNAS* 2011;108:4631-4638

LRR Sequence Classes

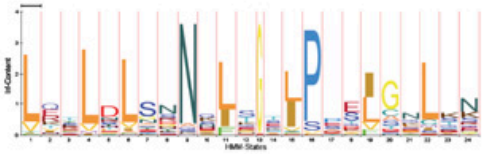
Bacterial (S) LxxLxVxxNxLxxLP(e/d)LzPxx



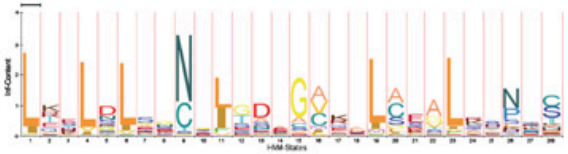
Cysteine-containing (CC) LxxLxLxxcxzxITDxxoxxLaxzxcxx



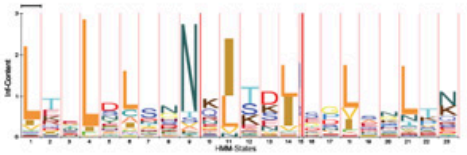
Plant-specific (PS) LxxLxLxxNxL(t/s)gzxIPxxLGxLxzx



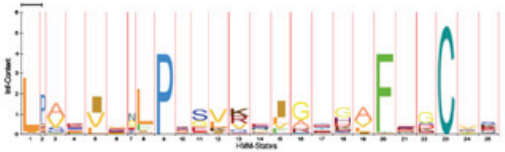
Ribonuclease inhibitor-like (RI) LxxLxLxx(N/C)xLxxxgoxxLxxoLxzxxxx



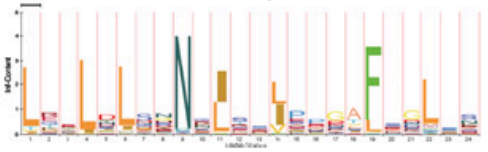
SDS22 LxxLxLxxNxlxxlxxLxzxLxx



Treponema pallidum (TP) LxxlxLxzxxLxxlgxxAFxx(C/N)xzx



Typical (T) LxxLxLxxNxLxxLpxxoFxzxLxx



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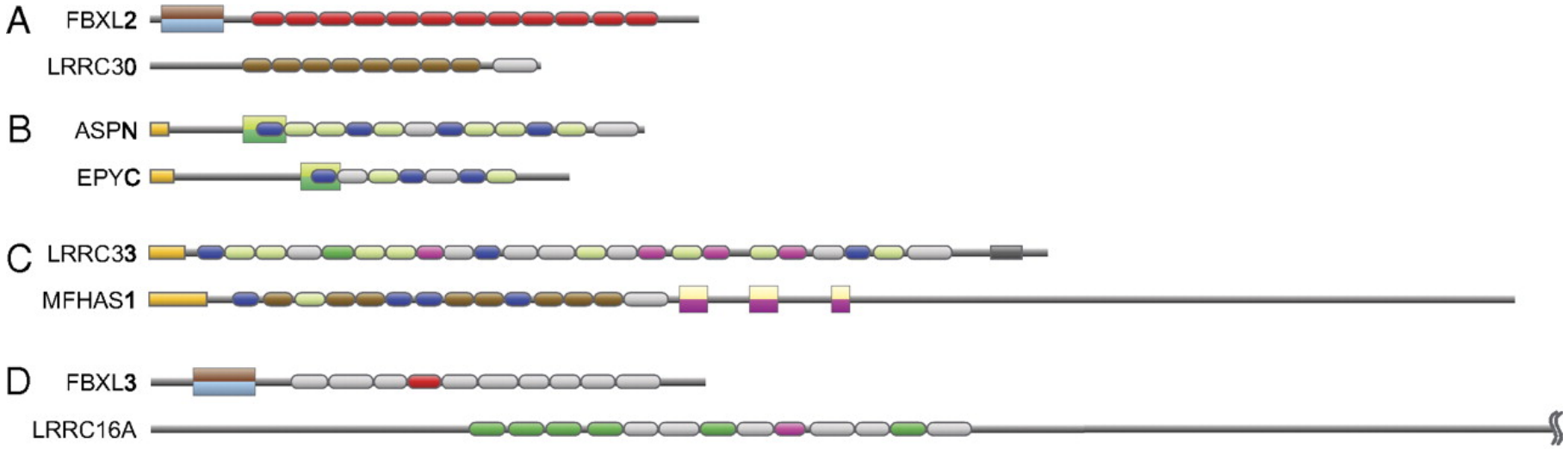
Examples of annotated LRR proteins.



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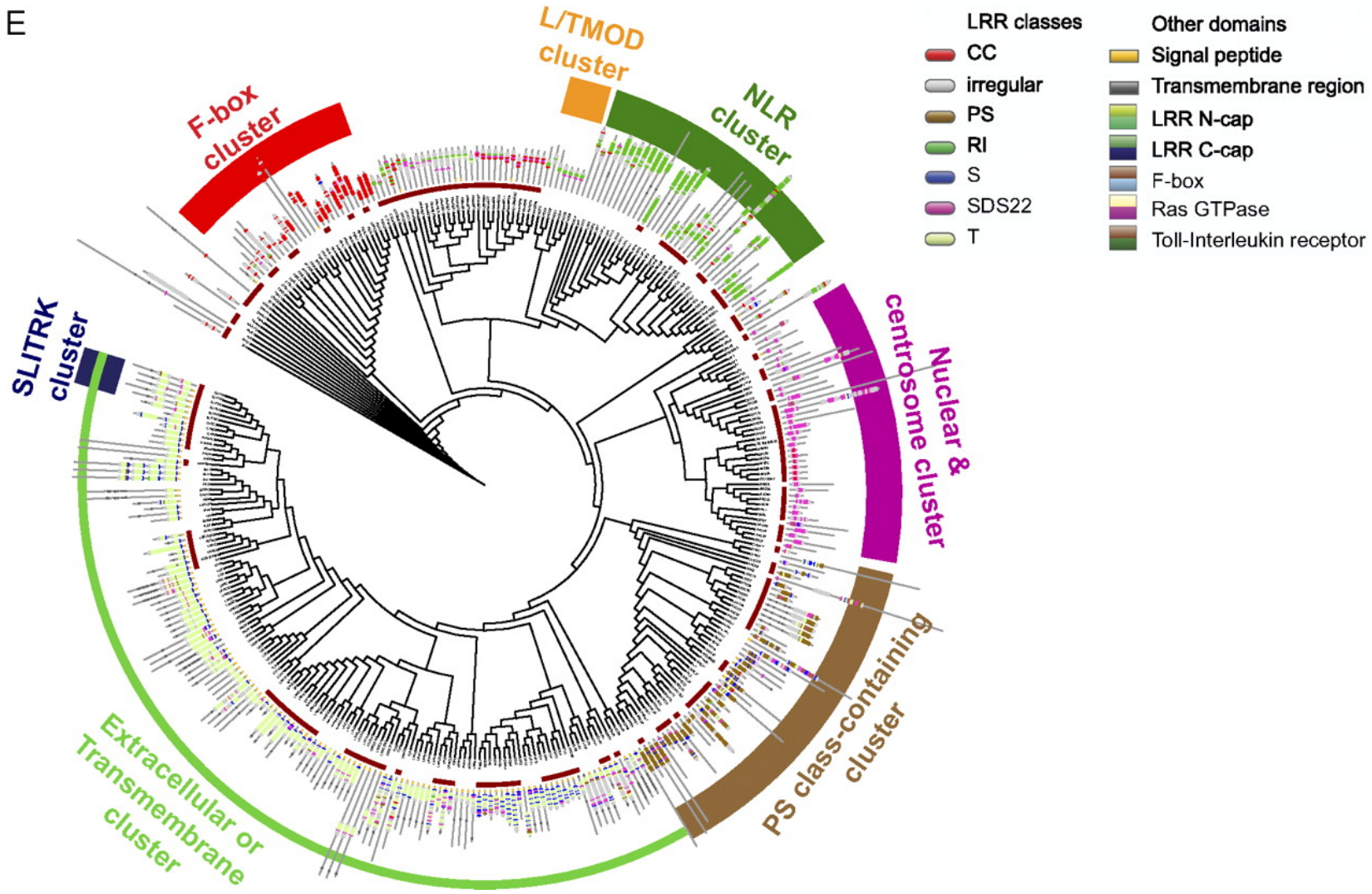
Clustering of annotated LRR proteins.



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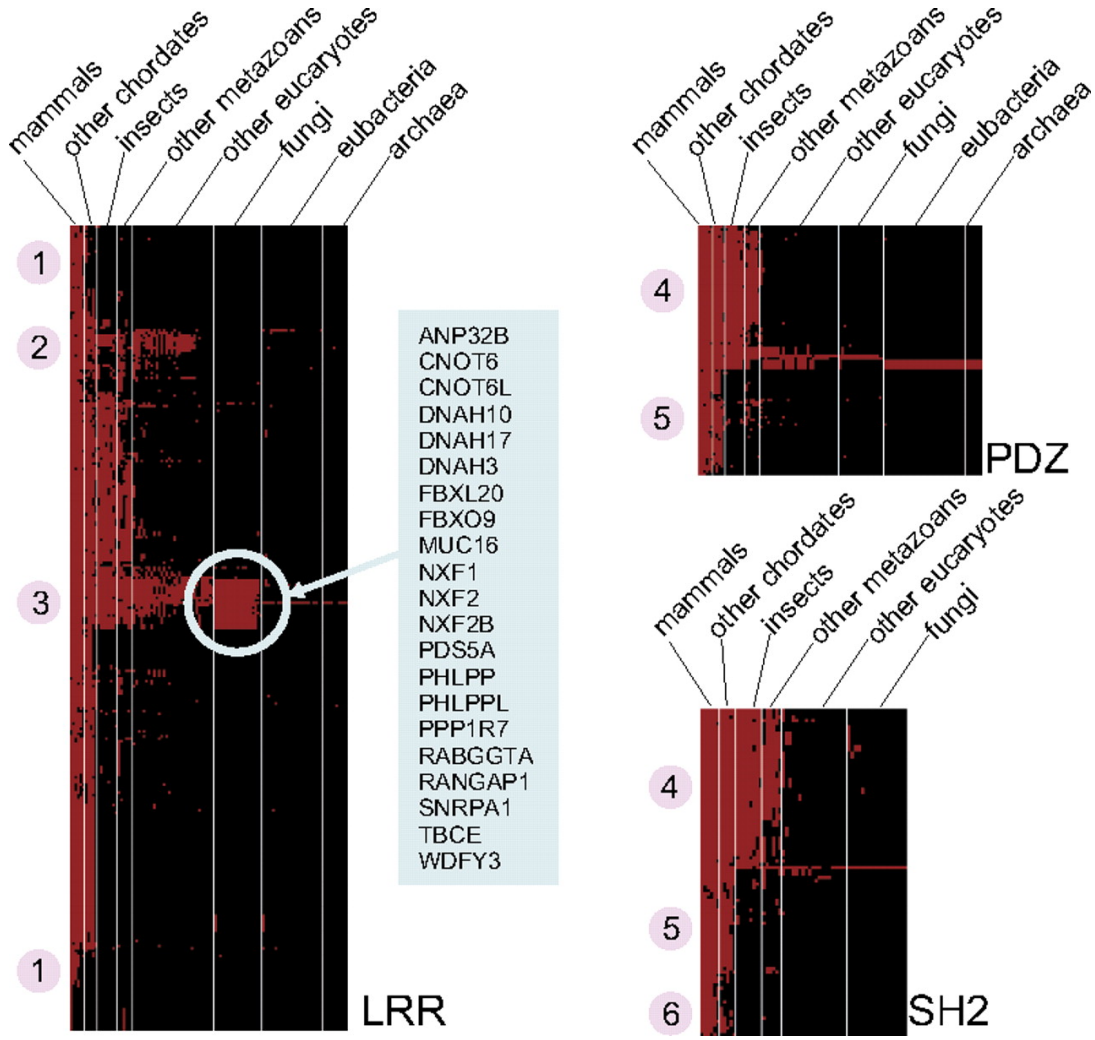
Heat maps indicating the species for which orthologues exist for human proteins containing LRR, PDZ, and SH2 domains.



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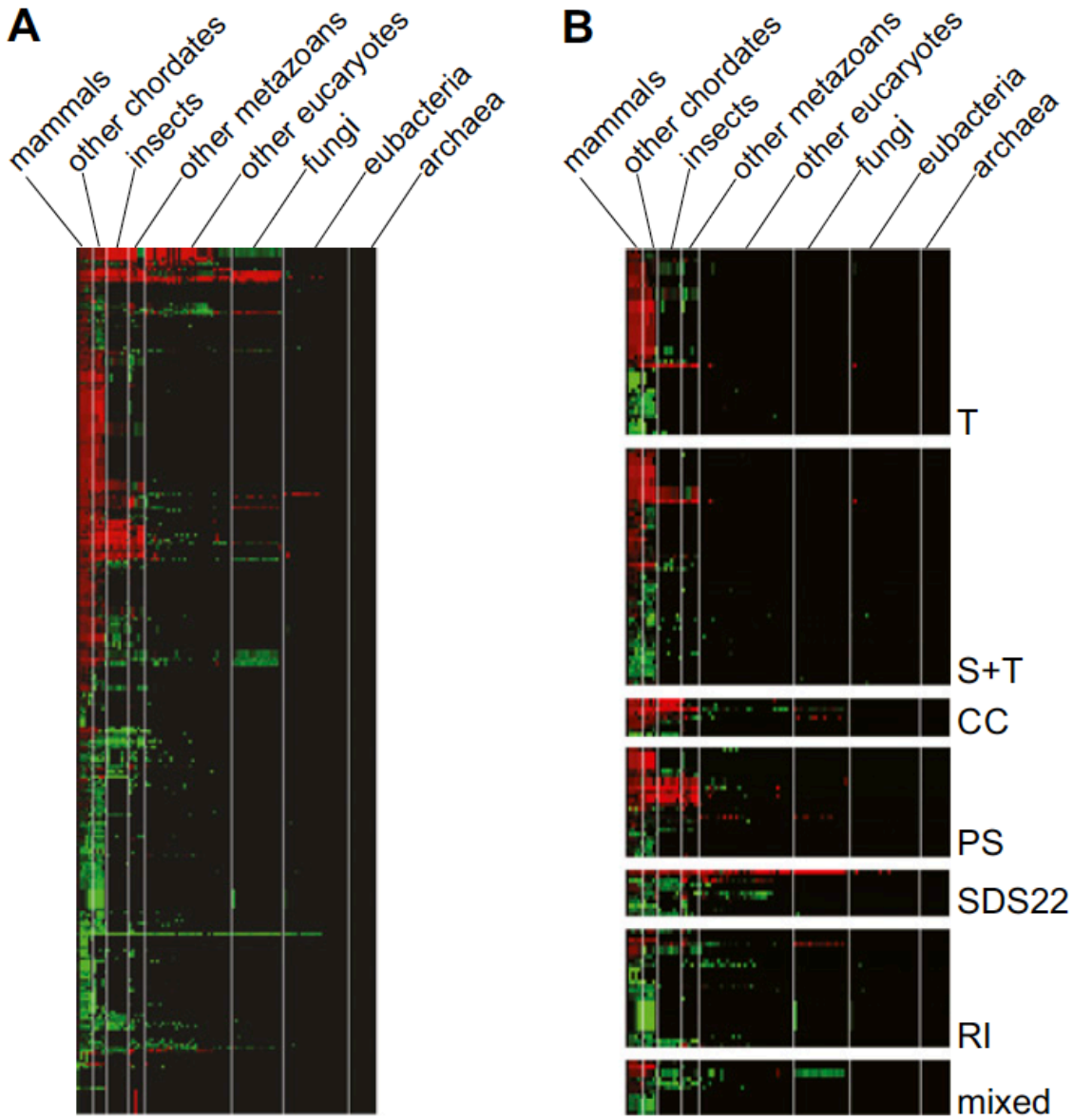
LRR Protein Conservation



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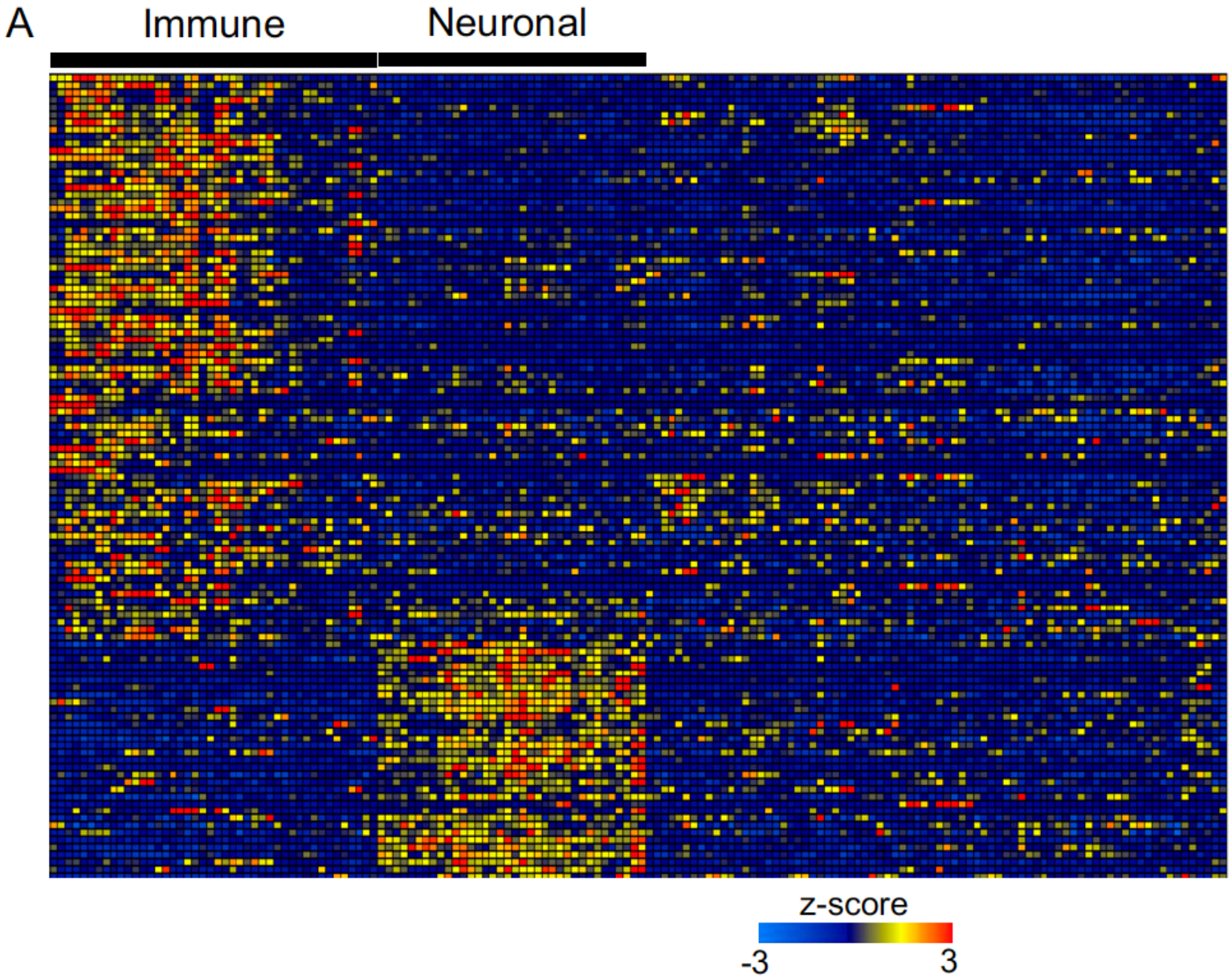
LRR mRNA Expression



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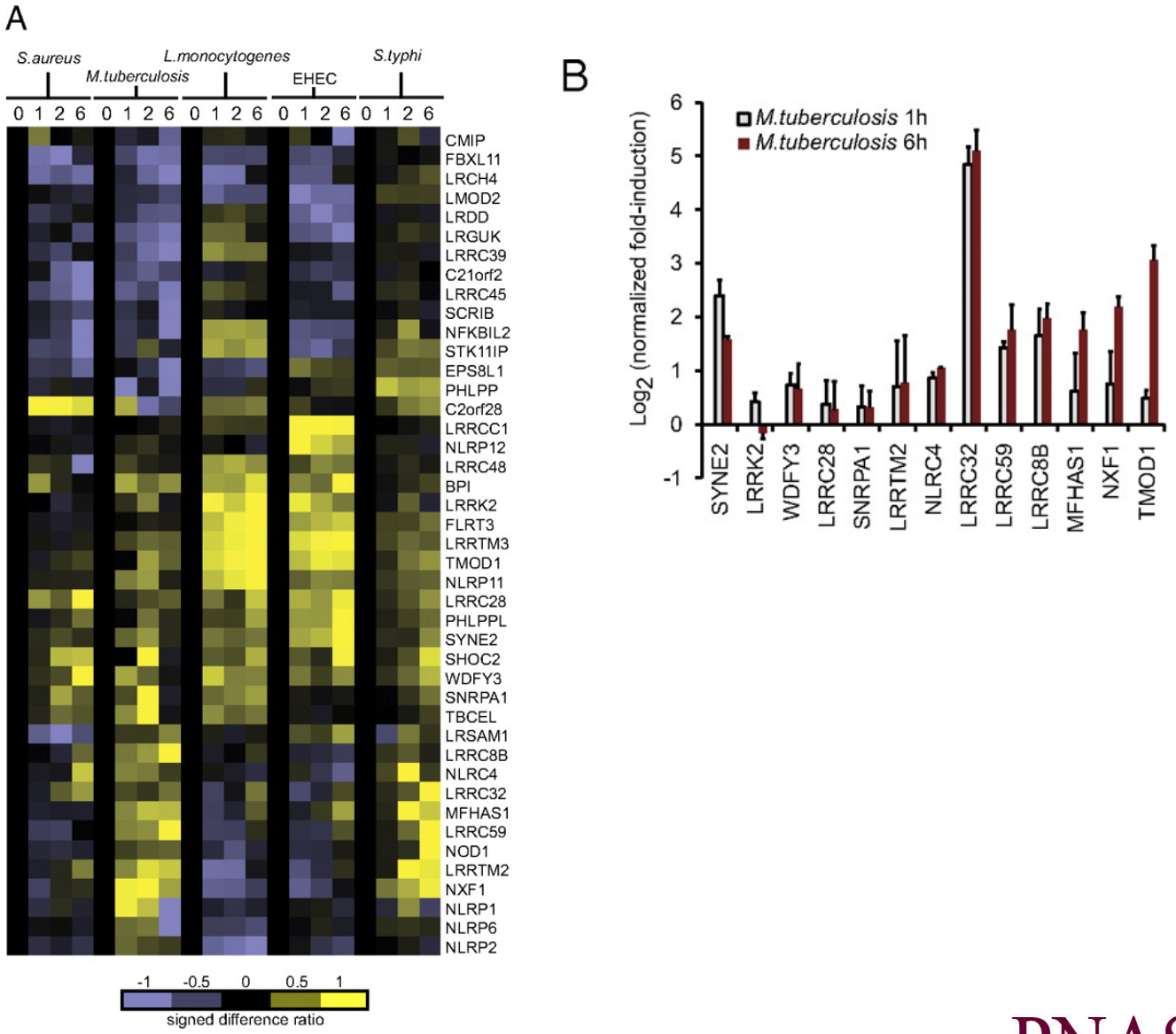
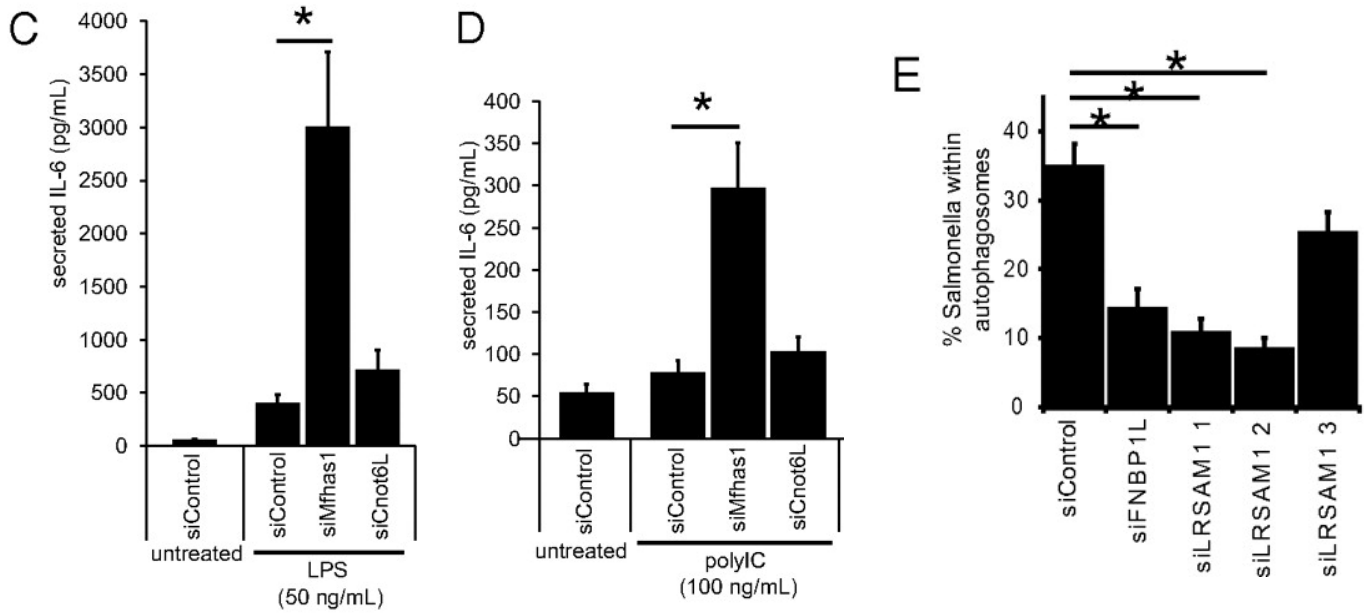
LRR proteins involved in immunity and antibacterial autophagy.



Ng A C Y et al. PNAS 2011;108:4631-4638

LRR proteins involved in immunity and antibacterial autophagy.



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LRR Domain Proteins: Conclusions and Questions

- Searching for LRR domains greatly expanded the list of proteins.
- A wide range of organisms express orthologous LRR proteins.
- Previously unknown LRR proteins influence inflammation and host defenses.
- The set of murine and human LRR proteins is not overlapping.
- What are the functions of LRR proteins within and between organisms?