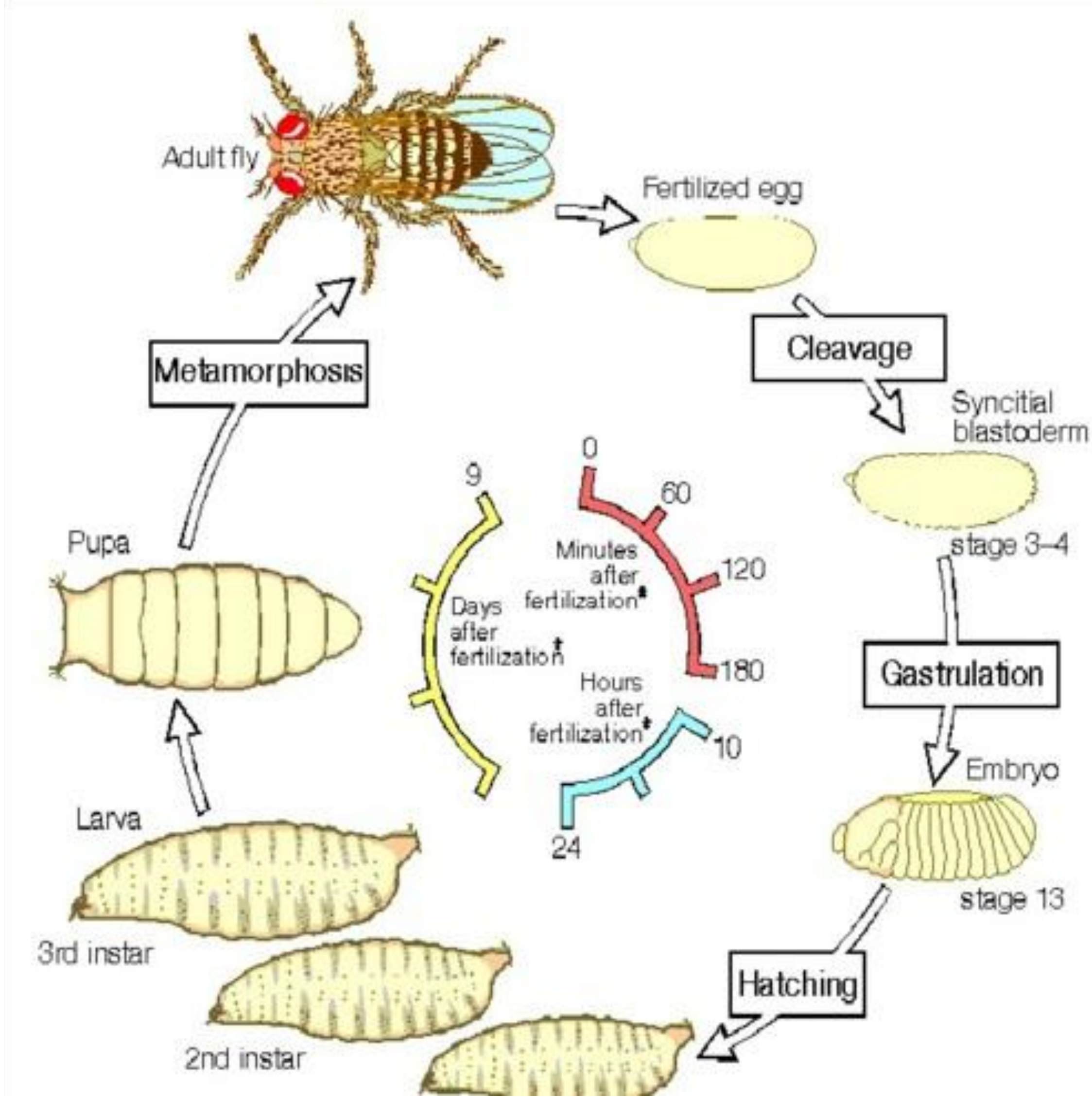
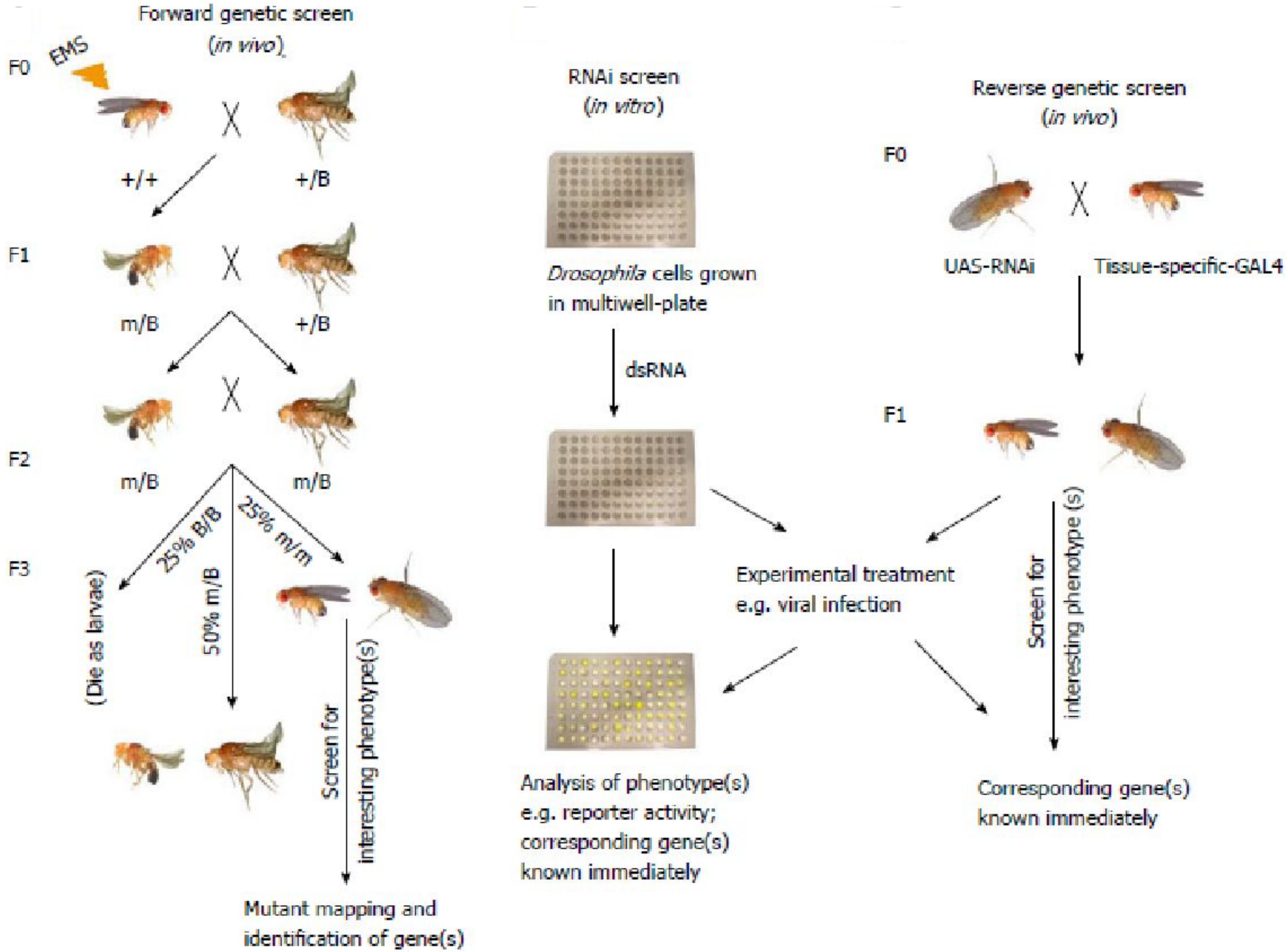
Эволюционная иммунология
Лекция 4 «Иммунитет дрозофилы»

Шилов Е.С.
5 марта МГУ, 2018

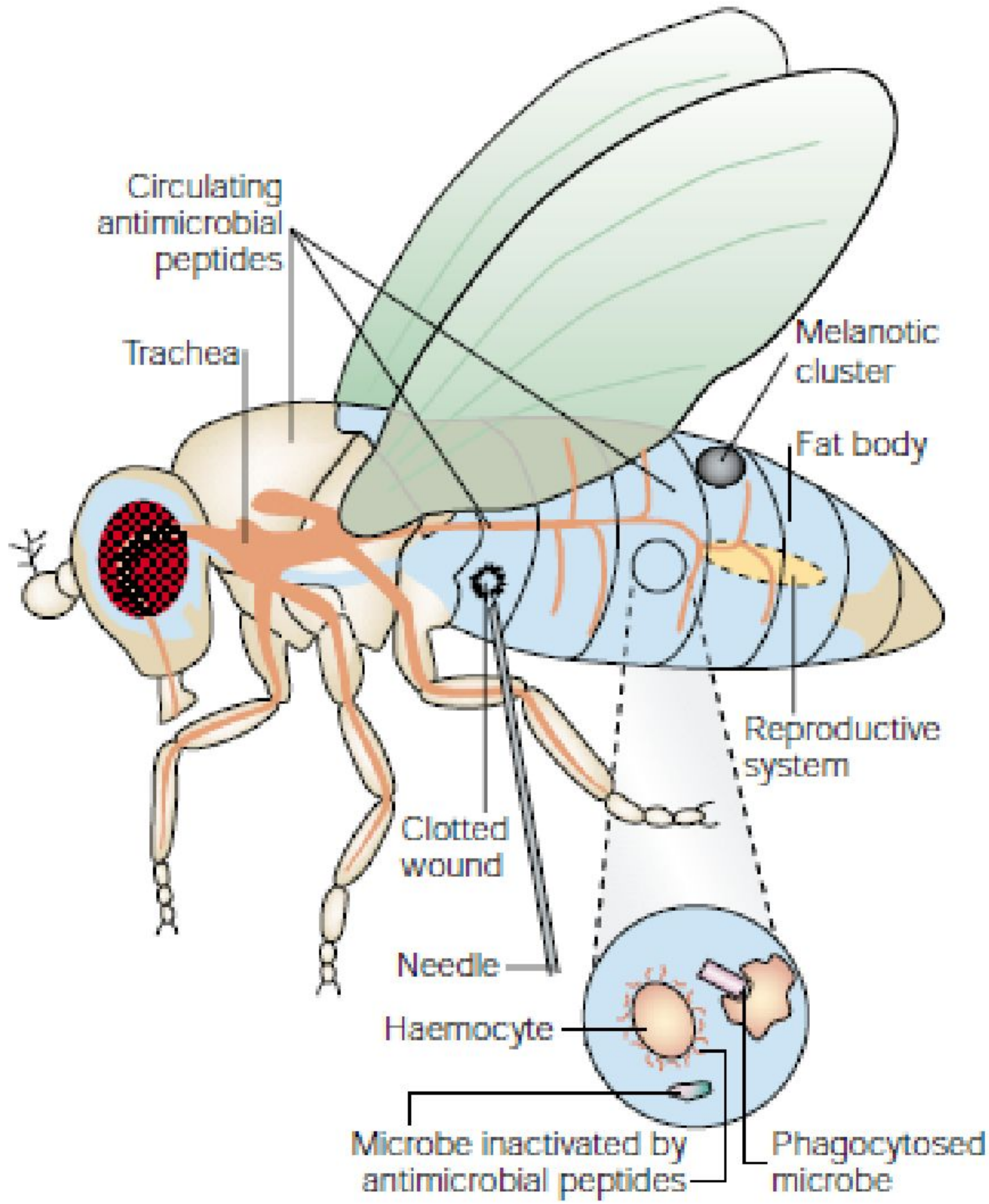
Жизненный цикл дрозофилы



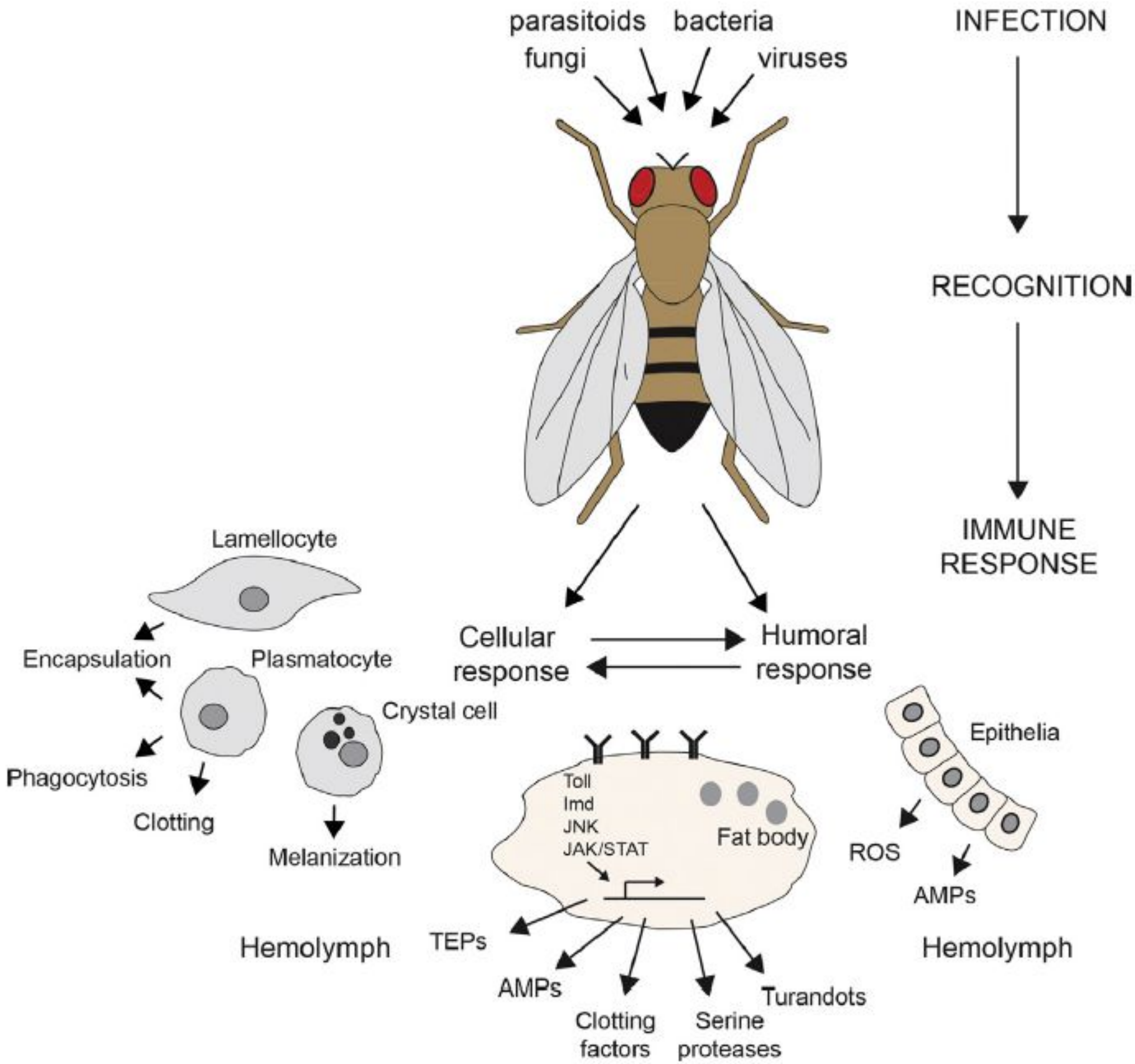
Генетические подходы, применимые к дрозофиле



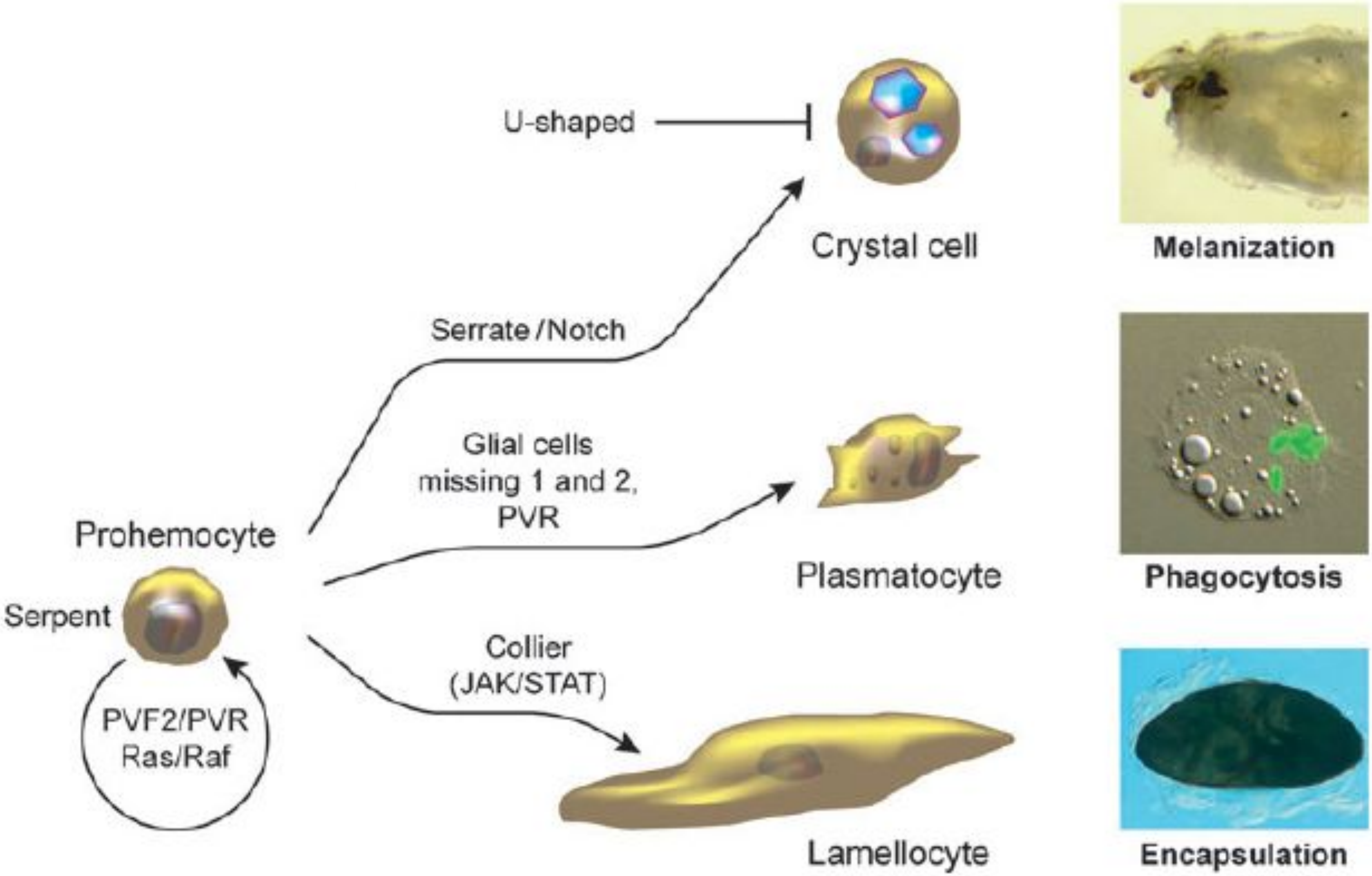
Иммунные реакции дрозофилы



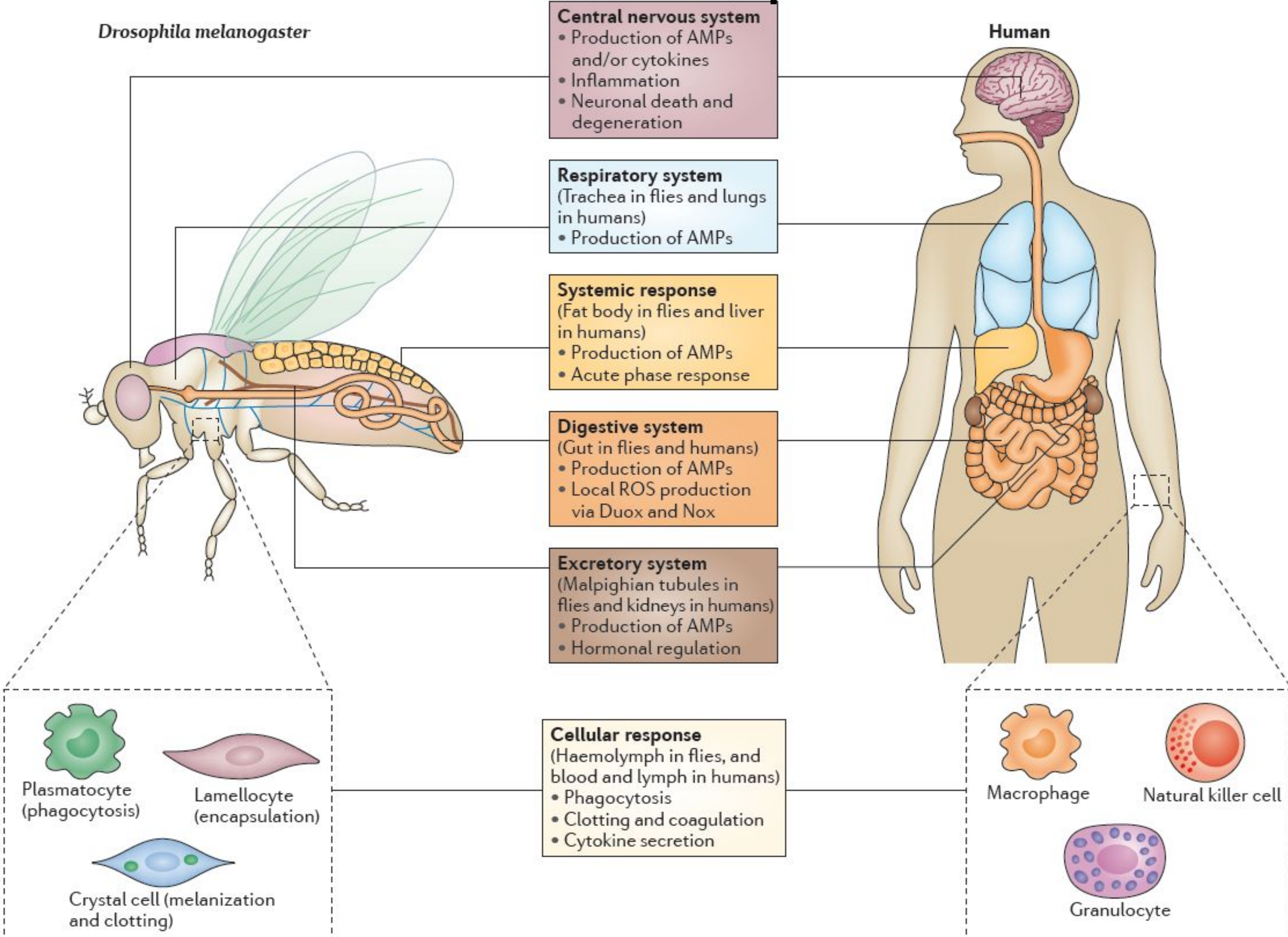
Разнообразие иммунных реакций дрозофилы



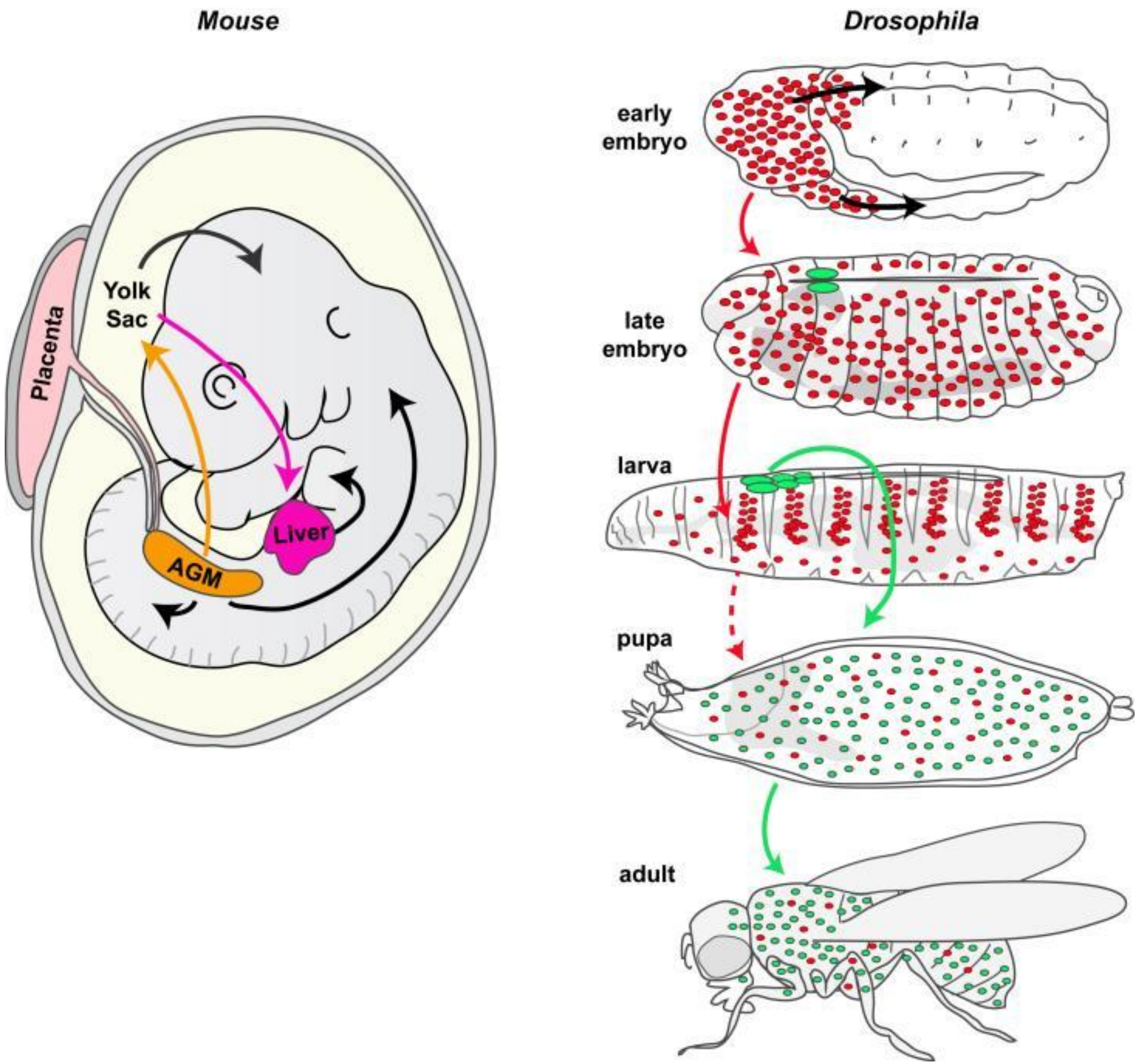
Типы иммунных клеток личинки дрозофилы



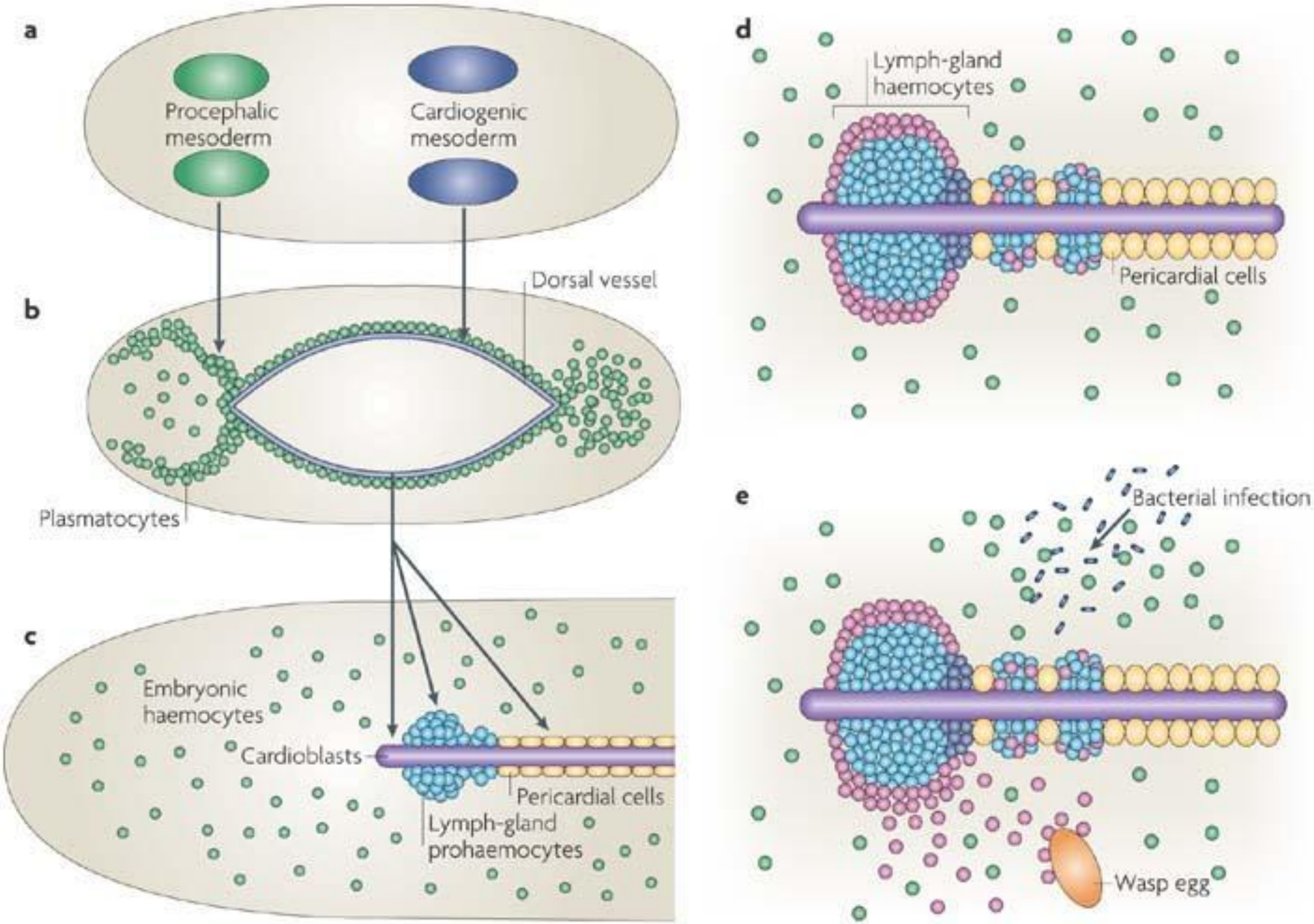
Сравнение иммунитета насекомых и млекопитающих



Сравнительный гематопоэз

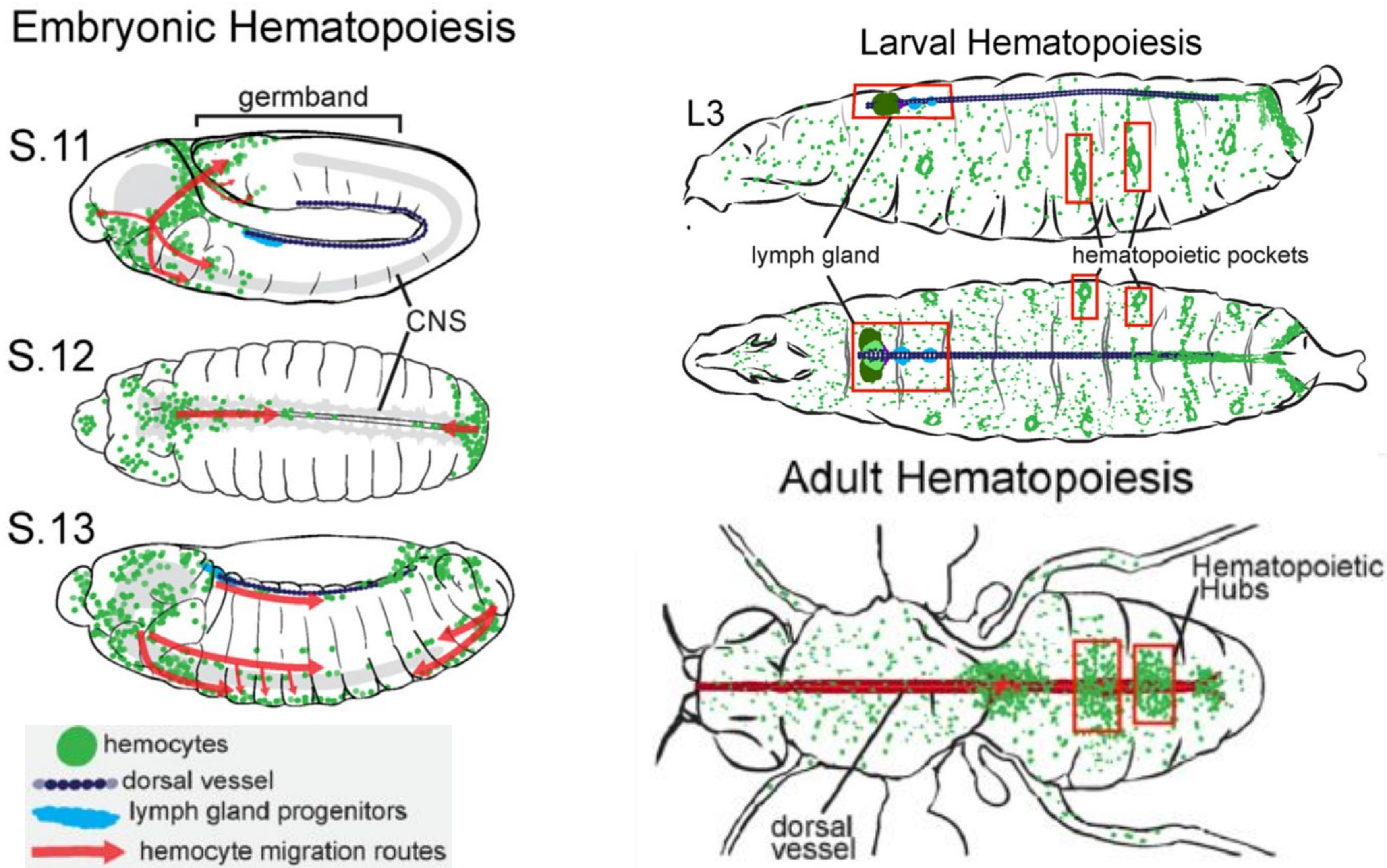


Происхождение гемоцитов дрозофилы

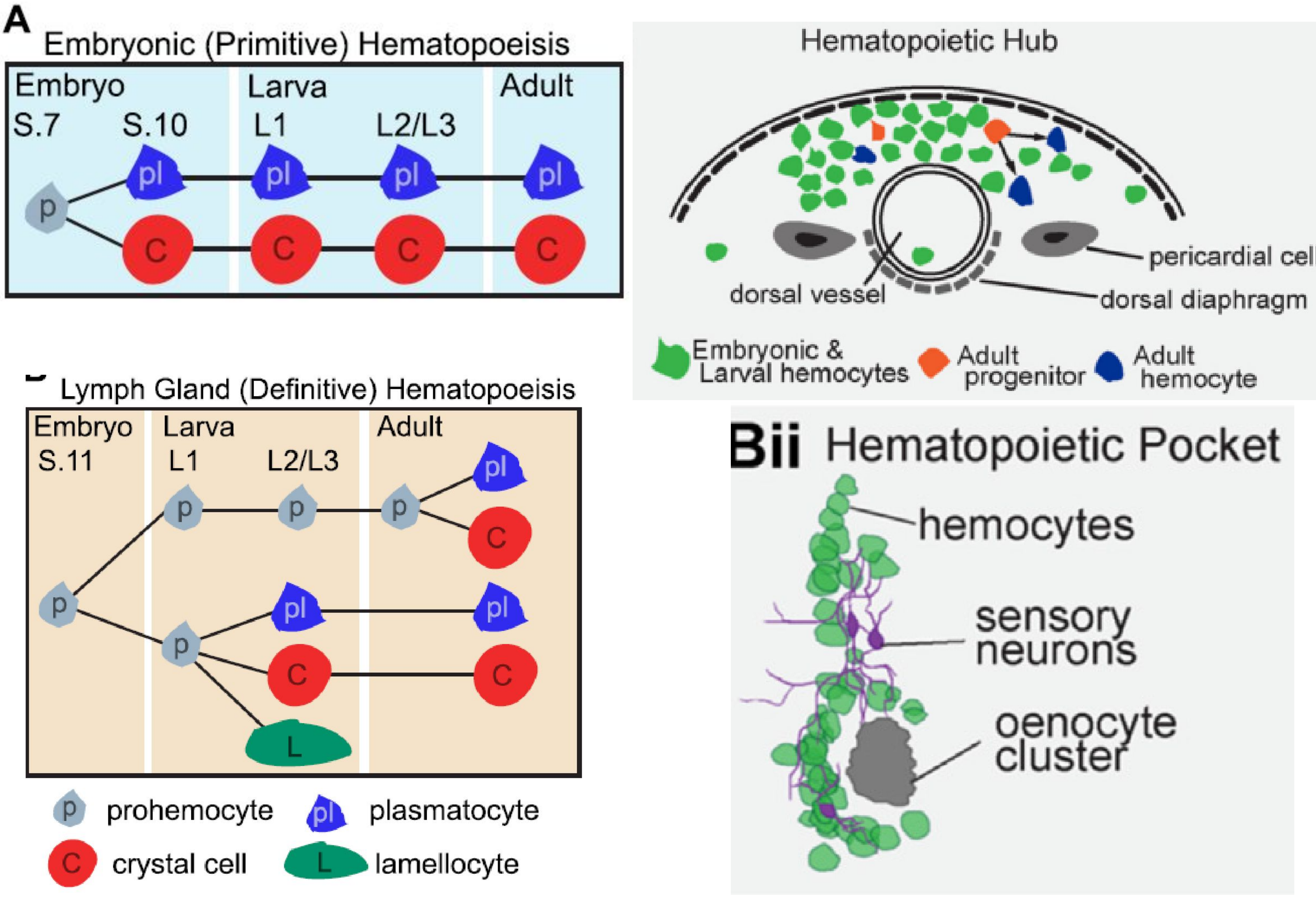


Nature Reviews | Molecular Cell Biology

Гематопоэз у имаго дрозофилы



Гематопоэз дрозофилы



Коагуляция гемолимфы у дрозофилы

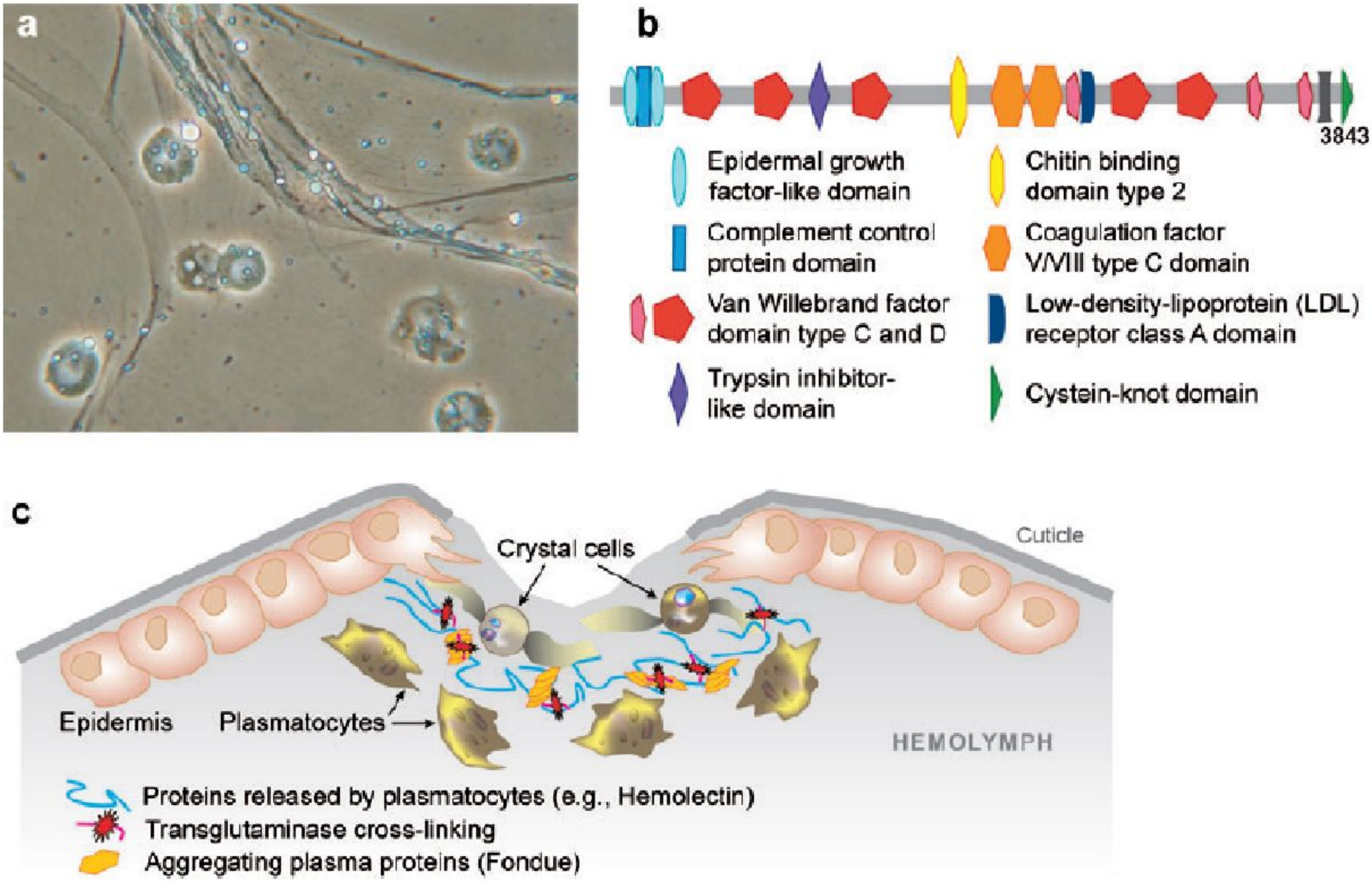
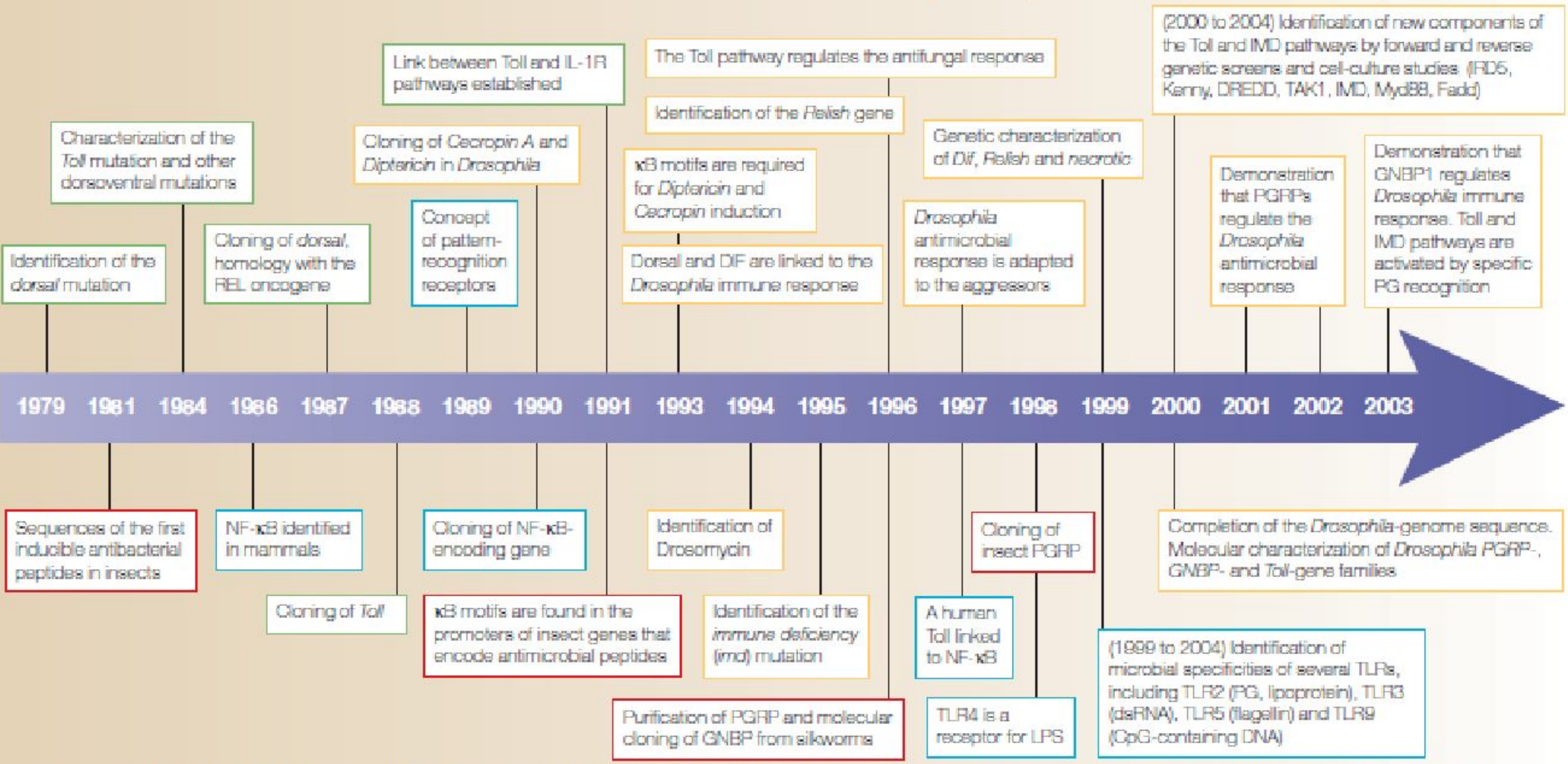


Table 1 | **Immune-related genes in *Drosophila* and mammals: homologies and analogies**

	Drosophila	Mammals*	References§
Haematopoietic determinants			
Transcription factors	*lozenge*	*AML1*	98
	serpent	Gata family	98
	gcm	Gcm family	98
Receptors			
Scavenger receptors (SR)	*epithelial membrane protein*	*CD36*	99
	SR-CI	Domains of *SRC1* have homology to various mammalian proteins; see also SR family	5,100
Gram-negative-binding proteins (GNBP)	GNBP family	Not identified	11
Peptidoglycan-recognition proteins (PGRP)	PGRP family	Pglyrp family	10
Lectins	*galactin*	Lgals family	55
Toll family	See text	See text	See text
Toll signalling components			
	dorsal, Dif, Rel	NF-κB family	17–19,101
	cactus	IκB family	102
	spätzle	*Bdnf*; homology is to cysteine knot nerve growth factors	103
	Spn43Ac of *necrotic* locus	*Spi17* and others that encode serine protease inhibitors of the serpin family	24
	gastrulation defective	*F2/prothrombin* and others that encode clotting factors	104,105
	pelle	*IRAK1*	106
	snake and *easter*	*Prss7* and *Klk/kallikrein* family	105
	IKK and *kenny*	IKK family	21,22
Signalling pathways other than Toll			
	hopscotch	JAK family	64
	Stat92E/marelle	STAT family	
	raf	*Raf1*	107
	basket	JNK family	108
	p38b	*p38*	109
Effectors			
Immunoglobulin motif	Various in *Drosophila*, but only reported insect immune related is *hemolin* of silkmoth	Immunoglobulin superfamily, including some *TIR*-related genes	110
Antimicrobial proteins	Antimicrobial protein gene families	Antimicrobial protein gene families	54,56,57,59
Metal ion accessibility and metalloproteinase regulation	*malvolio*	*Nramp/Scf11*	111
	Transferrin	*Transferrin*	112
	Other matrix metalloproteinase members, for example, *kuzbanian*	*Mmp7/matrilysin*	113
Translation related	*Thor*	4E-binding protein family	114
	lethal(1)aberrant immune response[8]	*ribosomal protein S6*	66
Cytotoxic molecules	Reactive intermediates of oxygen and nitrogen	Reactive intermediates of oxygen and nitrogen	115
Apoptosis and immunity			
	croquemort	*CD36*	6
	dredd	CASP family	90–92
Additional components			
	Tep family	*C3* and *A2M*	65,72
	Various from genome annotation	For example, genes that encode C1 and fibrinogen domains	13,84

Хронология изучения иммунитета мушки

Timeline | **Discoveries relevant to the function of the Toll and Imd pathways in *Drosophila* immunity**



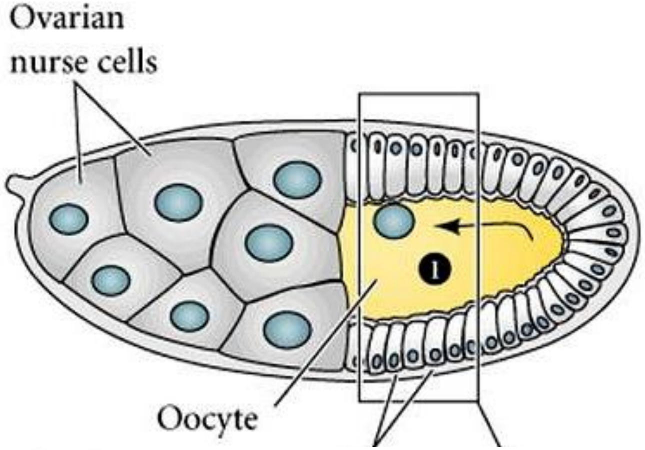
The discovery of the role of Toll in the *Drosophila* immune response (yellow) was influenced mainly by research in three fields: insect immunity (red), vertebrate immunology and signalling (blue) and developmental genetics (green). DIF, Dorsal-related immunity factor; DREDD, Death-related ced-3/Nedd2-like protein; ds, double-stranded; GNBP, Gram-negative-bacteria-binding protein; IκB, inhibitor of NF-κB; IKK, IκB kinase; IL-1R, interleukin-1 receptor; IMD, Immune deficiency; IRD5, Immune-response deficient 5; LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; PG, peptidoglycan; PGRP, PG-recognition protein; serpin, serine-protease inhibitor; TAK1, Transforming growth factor-β-activated kinase 1; TLR, Toll-like receptor.

Christiane Nüsslein-Volhard

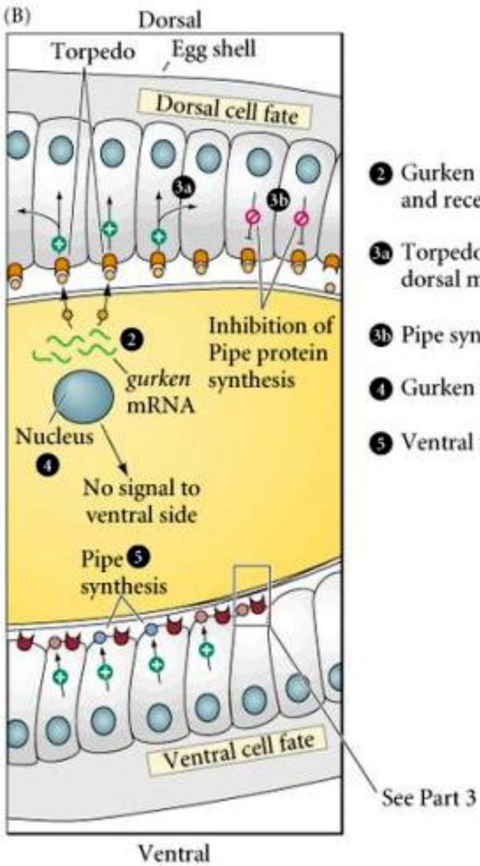
Kathryn V. Anderson

Lemaitre Bruno

Jules A. Hoffmann



(B)



2 Gurken protein made by oocyte and received by Torpedo protein

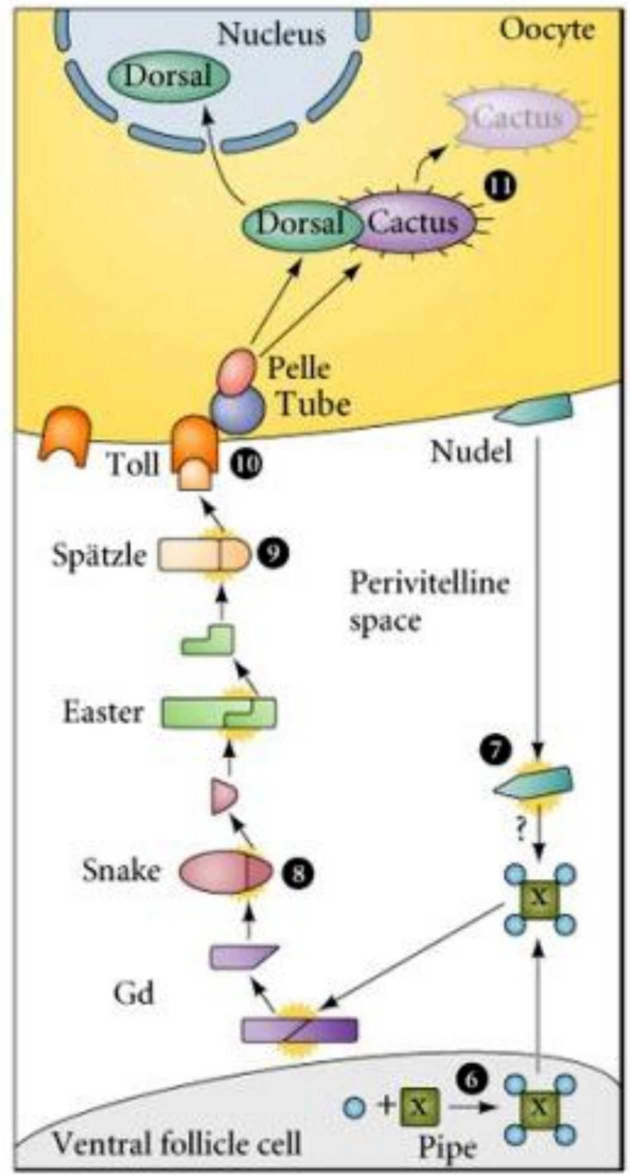
3a Torpedo causes dorsal morphology

3b Pipe synthesis inhibited

4 Gurken does not diffuse

5 Ventral follicle cells make Pipe

(C)



6 Pipe modifies (x)

7 Nudel and (x) activate Gd

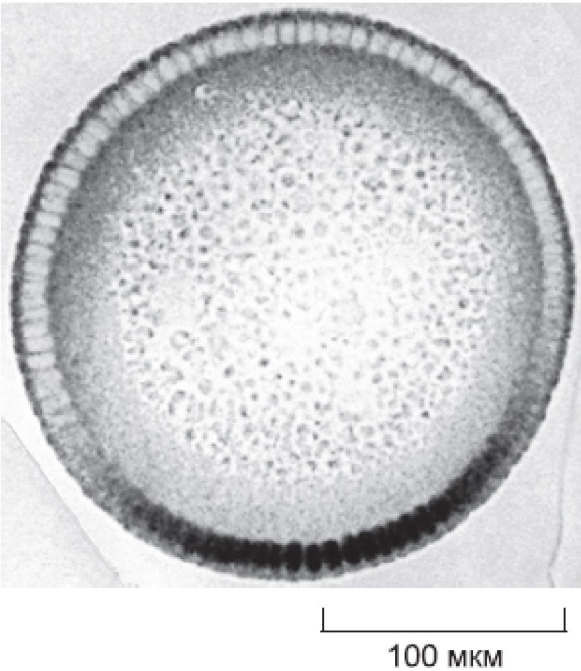
8 Gd activates Snake; Snake activates Easter

9 Easter activates Spätzle, which binds to Toll

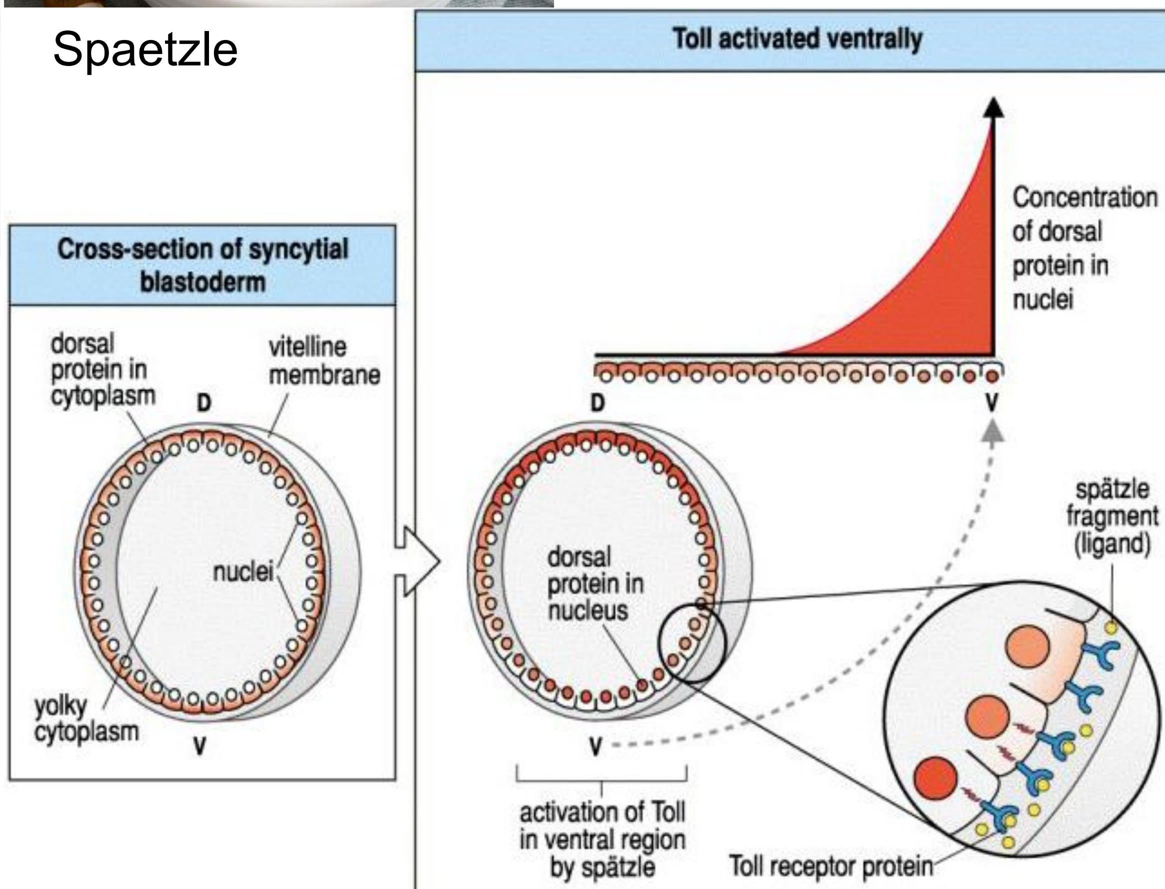
10 Toll activates Tube and Pelle, to phosphorylate Cactus

11 Cactus is degraded; Dorsal protein enters nucleus

Белок Dorsal в верхней части зародыша мухи попал в ядра, в нижней части – остался в цитоплазме



Spaetzle



NF-κB у дрозофилы: Dorsal, DIF, Relish

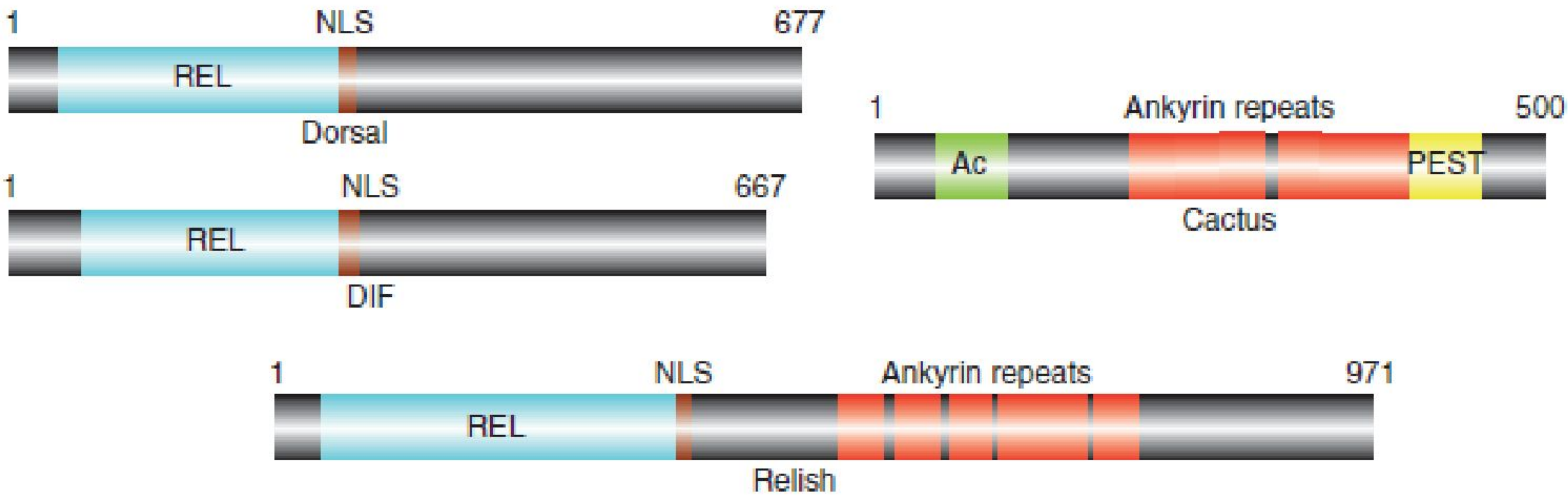
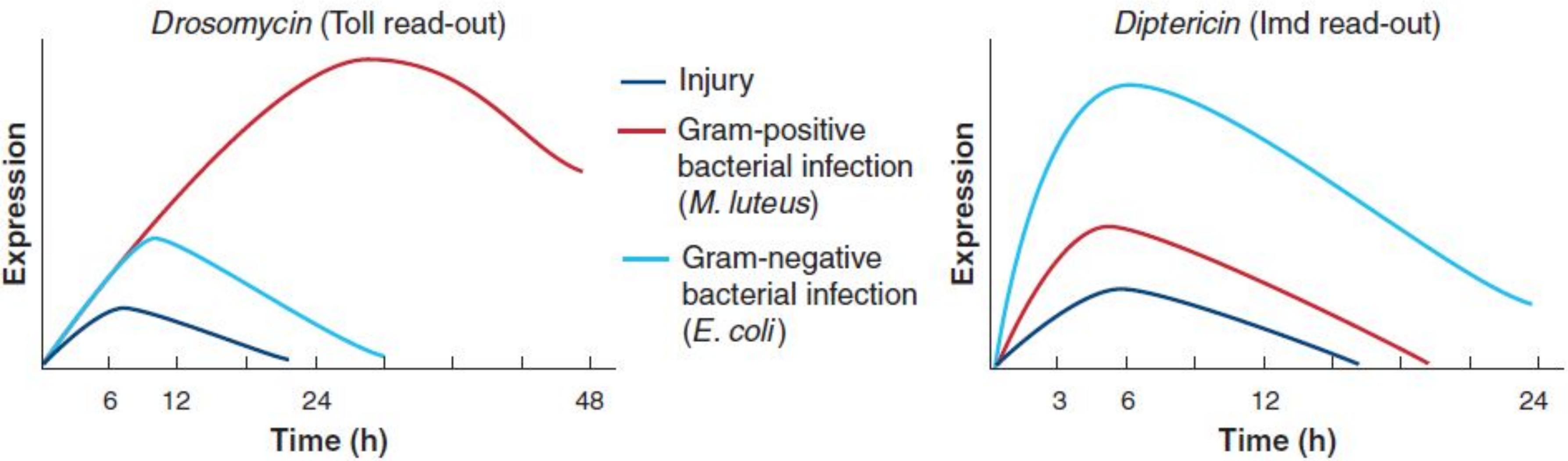
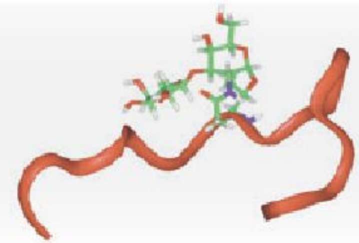
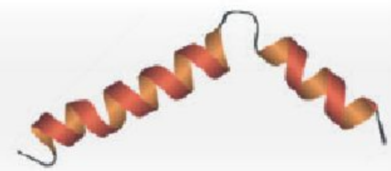
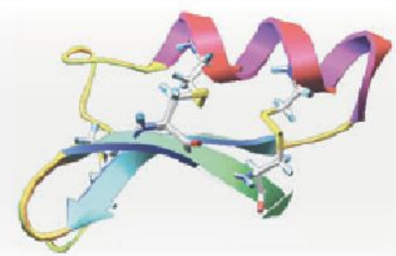
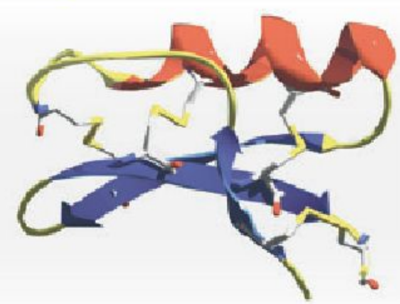


Figure 1. The NF-κB and IκB proteins in *Drosophila*. The length in amino acids is indicated by numbers. REL, Rel-homology domain; NLS, nuclear localization sequence; PEST, proline, glutamic acid, serine, and threonine-rich segment; Ac, acidic domain.

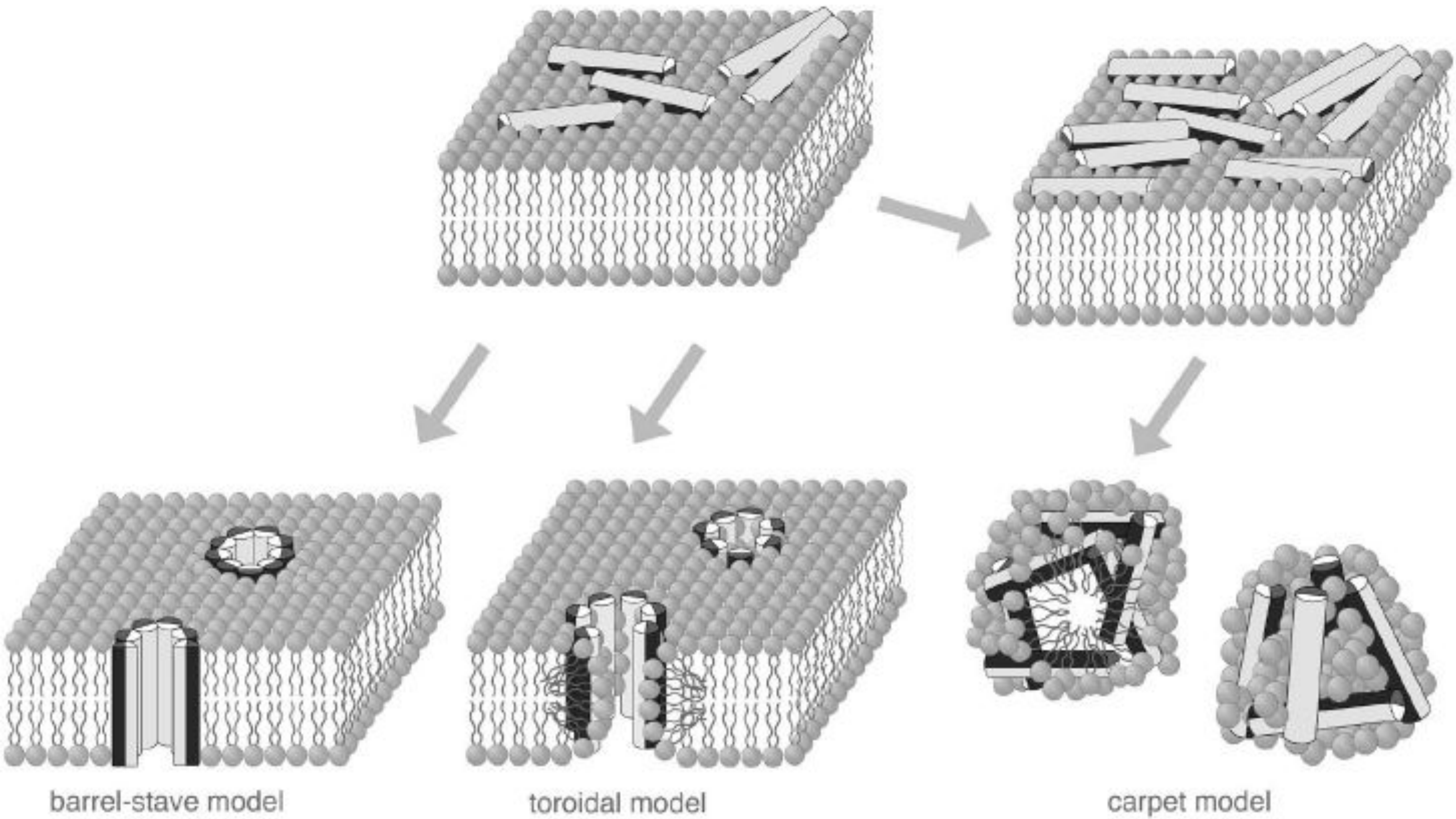
Грамотрицательные и грамположительные бактерии запускают соответственно Imd- и TLR- пути, которые различаются по спектру активируемых антибактериальных пептидов



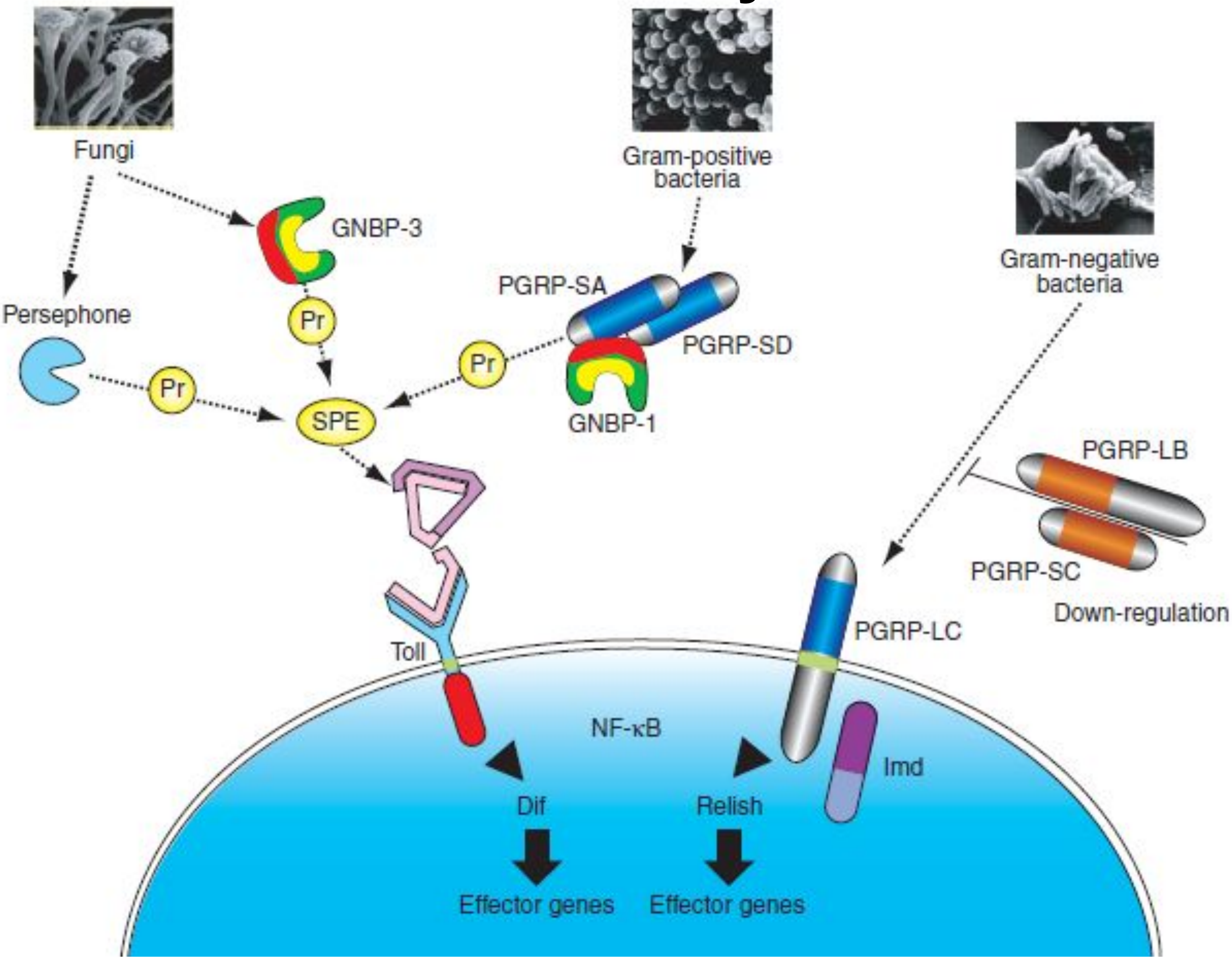
Семь семейств антимикробных пептидов дрозофилы

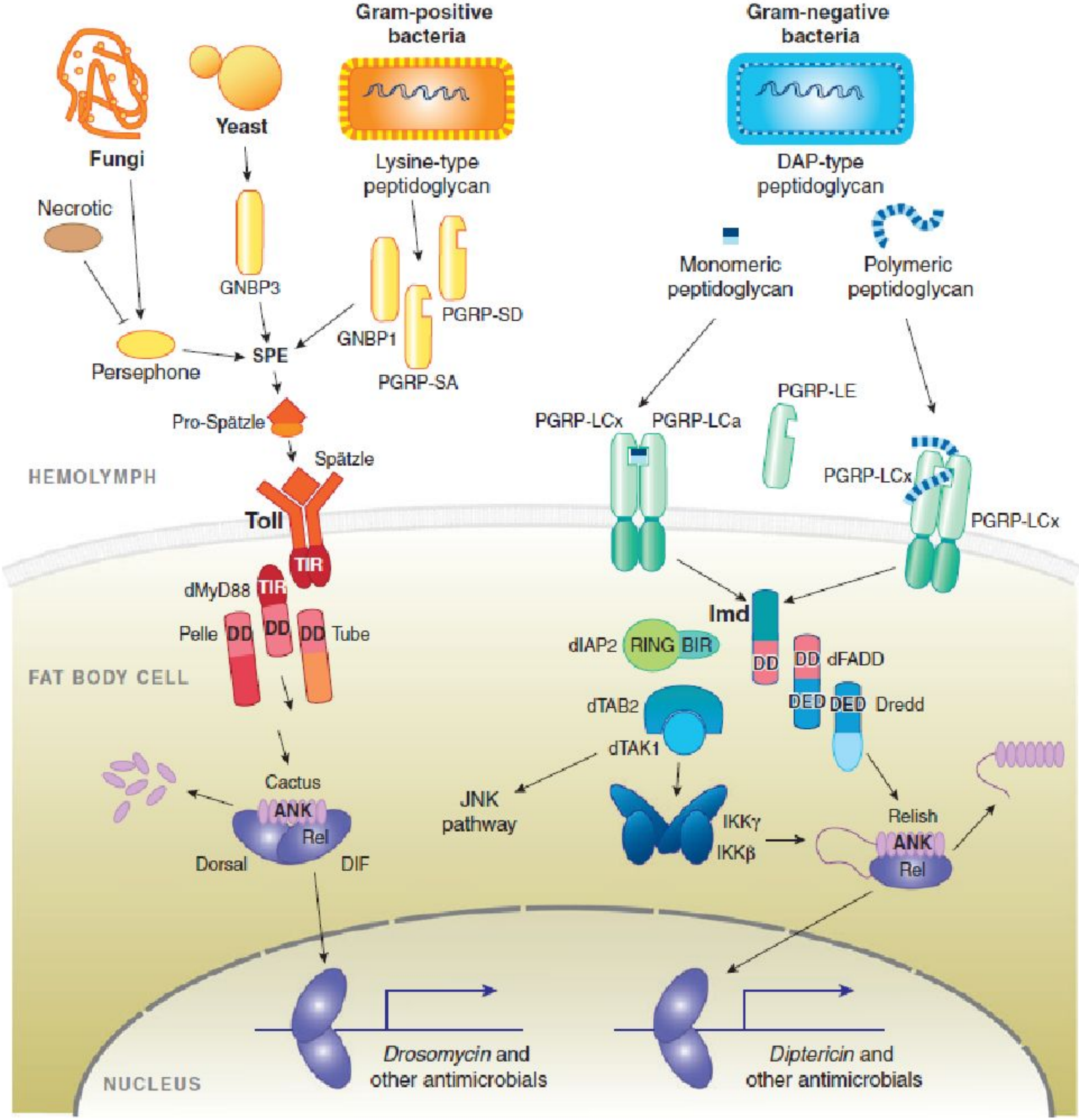
Peptides	# of genes	Main activity	Concentration	3-D structure
Diptericin	2	Gram-negative bacteria	0.5 μM	nd
Attacin	4	Gram-negative bacteria	nd	nd
Drosocin	1	Gram-negative bacteria	40 μM	
Cecropin	4	Gram-negative bacteria	20 μM	
Defensin	1	Gram-positive bacteria	1 μM	
Drosomycin	7	Fungi	100 μM	
Metchnikowin	1	Fungi	10 μM	nd

Механизмы встраивания АМП в мембрану патогена

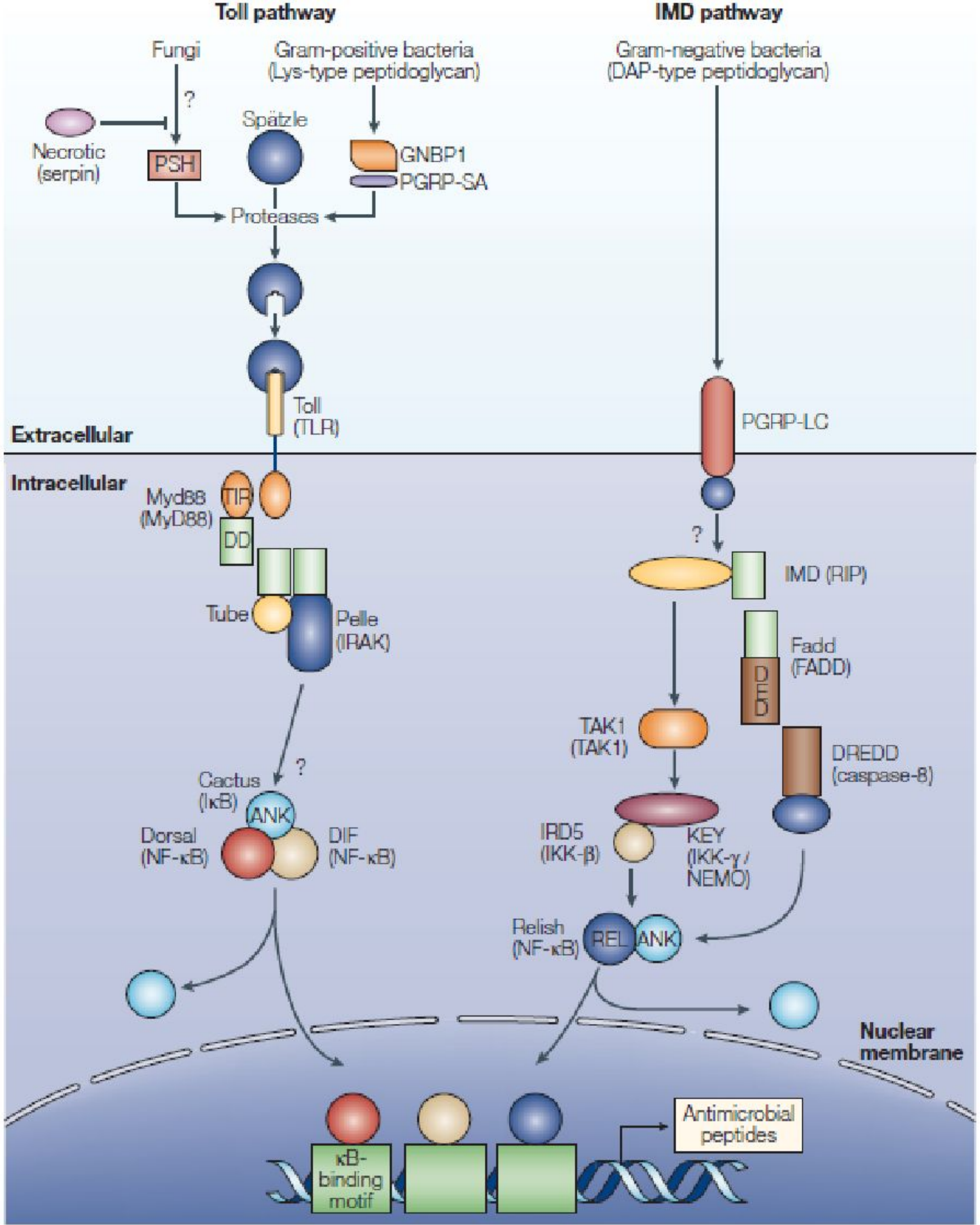


Toll и Imd пути

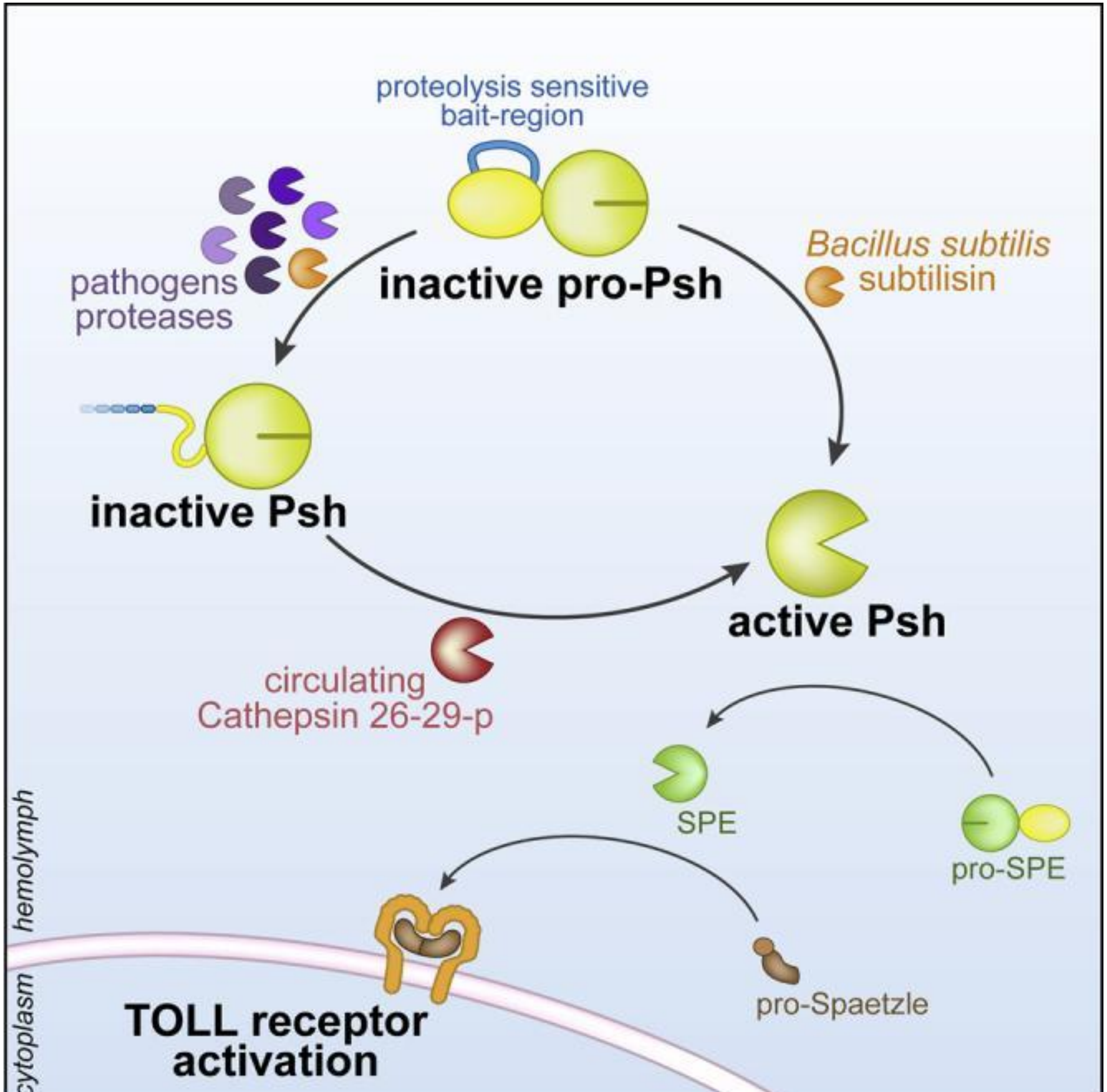




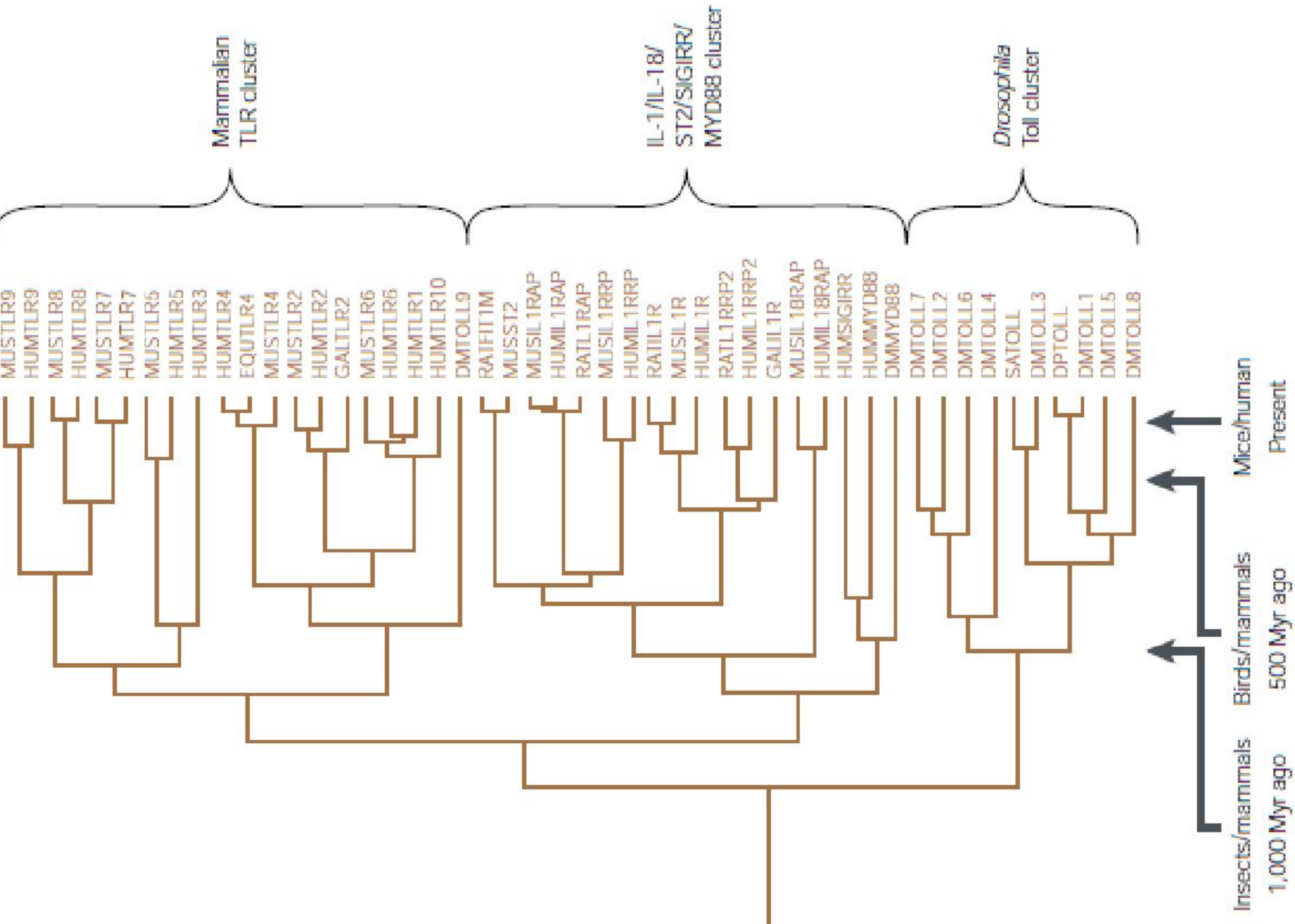
Gram-positive bacteria
Gram-negative bacteria
Yeast
Fungi
Lysine-type peptidoglycan
DAP-type peptidoglycan
Necrotic
GNBP3
PGRP-SD
GNBP1
PGRP-SA
Persephone
SPE
Monomeric peptidoglycan
Polymeric peptidoglycan
Pro-Spätzle
Spätzle
PGRP-LCx
PGRP-LCa
PGRP-LE
PGRP-LCx
PGRP-LCx
HEMOLYMPH
Toll
TIR
dMyD88
TIR
Pelle
DD
DD
DD
Tube
FAT BODY CELL
Imd
dIAP2
RING
BIR
DD
DD
dFADD
dTAB2
DED
DED
Dredd
dTAK1
Cactus
ANK
Rel
Dorsal
DIF
JNK pathway
IKKγ
IKKβ
Relish
ANK
Rel
Drosomycin and other antimicrobials
Diptericin and other antimicrobials
NUCLEUS

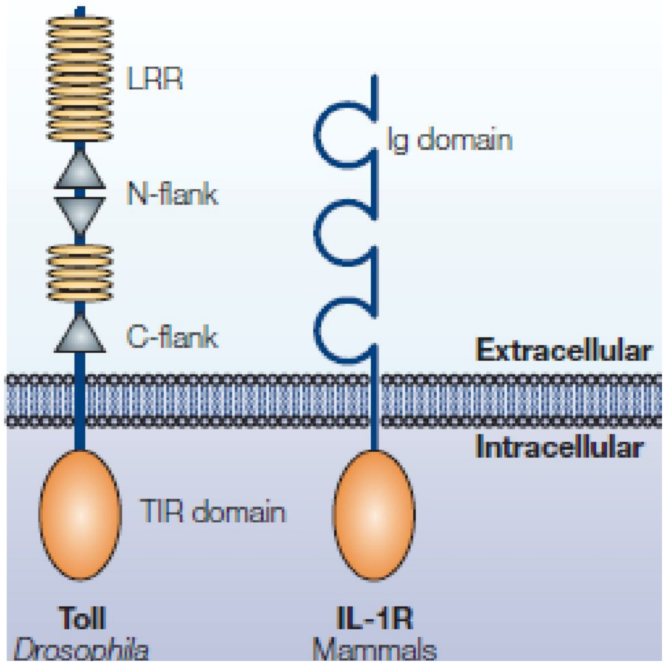

Toll pathway
IMD pathway
Fungi
Gram-positive bacteria
(Lys-type peptidoglycan)
Gram-negative bacteria
(DAP-type peptidoglycan)
?
Necrotic
(serpin)
PSH
Spätzle
GNBP1
PGRP-SA
Proteases
Toll
(TLR)
PGRP-LC
Extracellular
Intracellular
Myd88
(MyD88)
TIR
DD
?
IMD (RIP)
Tube
Pelle
(IRAK)
Fadd
(FADD)
DED
TAK1
(TAK1)
DREDD
(caspase-8)
?
Cactus
(IκB)
ANK
Dorsal
(NF-κB)
DIF
(NF-κB)
IRD5
(IKK-β)
KEY
(IKK-γ /
NEMO)
Relish
(NF-κB)
REL
ANK
Nuclear
membrane
κB-
binding
motif
Antimicrobial
peptides

Персефона активируется бактериальными протеазами

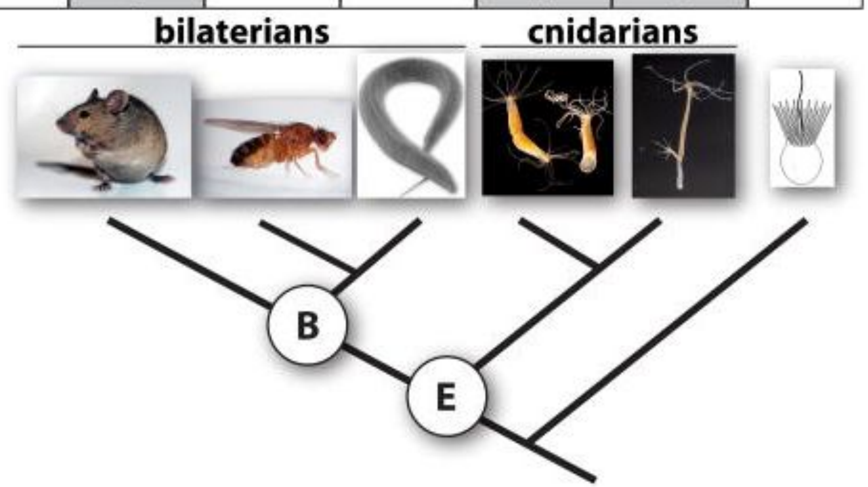


Эволюция Toll-подобных рецепторов

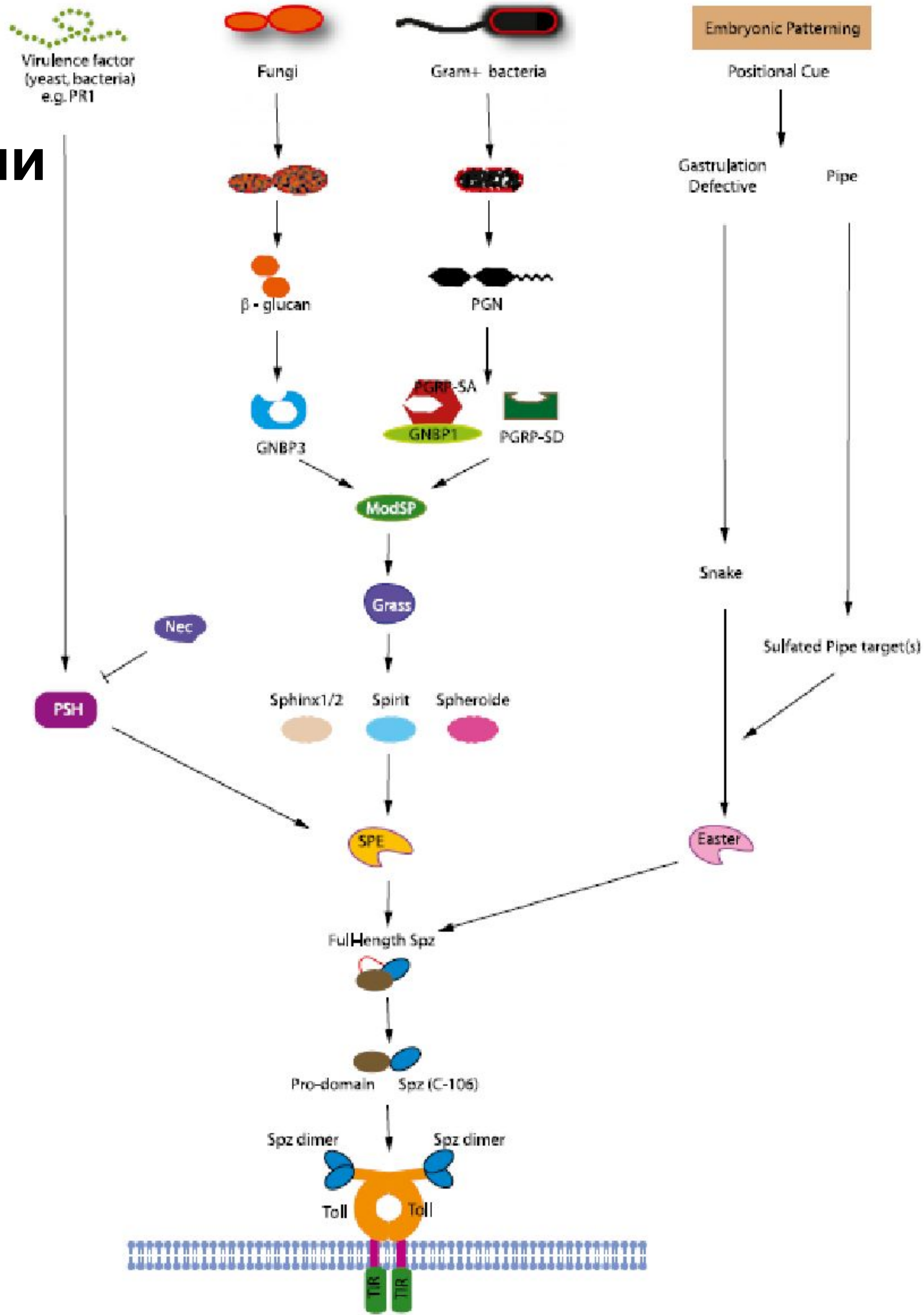




	Mus musculus	Drosophila melanogaster	Caenorhabditis elegans	Nematostella vectensis	Hydra magnipapillata	Monosiga brevicollis
TLR	+	+	+	+	?	
SARM	+	+	+			
MyD88	+	+		+	+	
IkB	+	+	+	+	+	
NF-kB	+	+		+		
p38 MAPK	+	+	+	+	+	+
NLR	+			+	?	

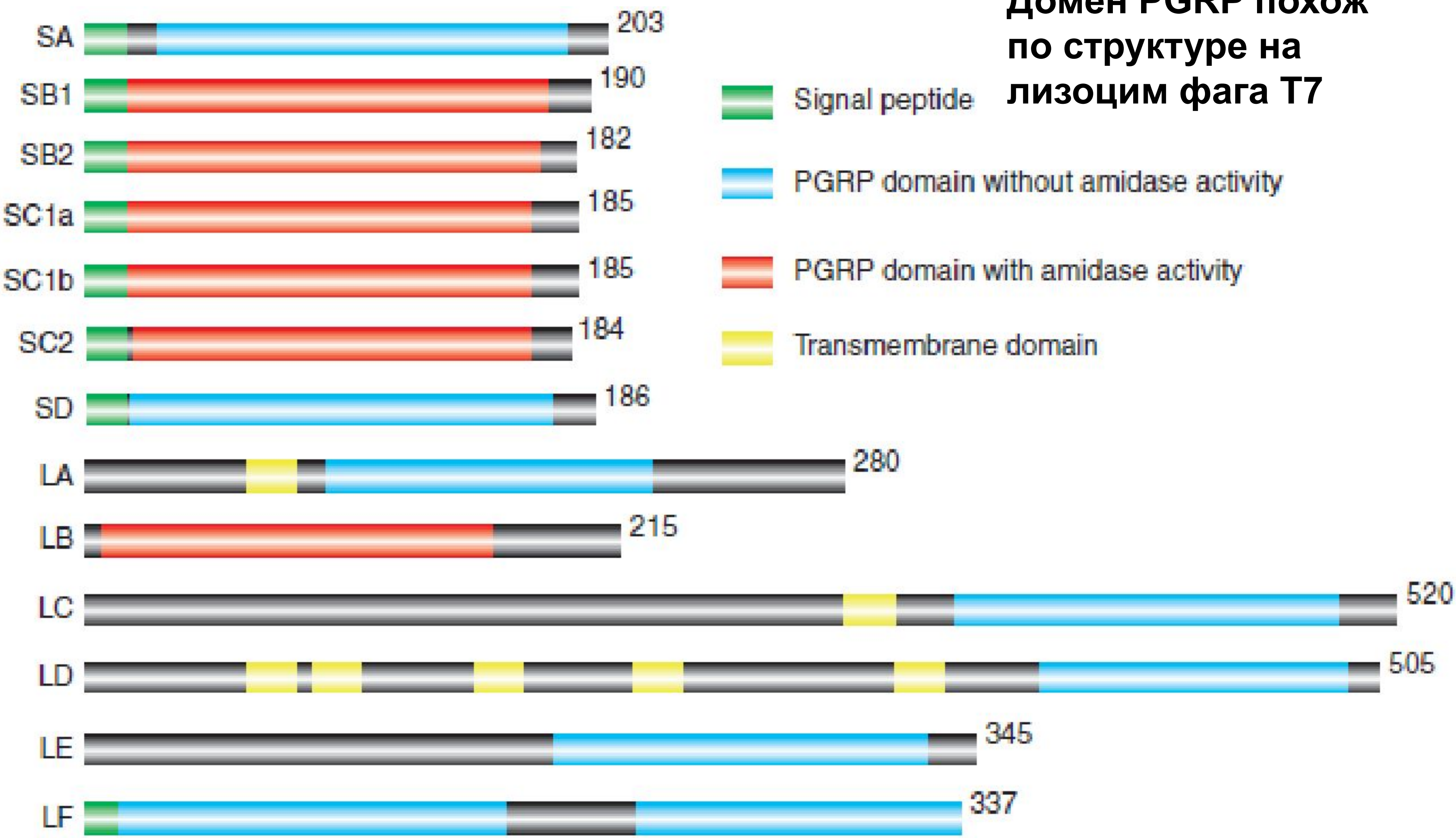


Первичными рецепторами PAMP в Toll-пути у дрозофилы является множество различных белков гемолимфы

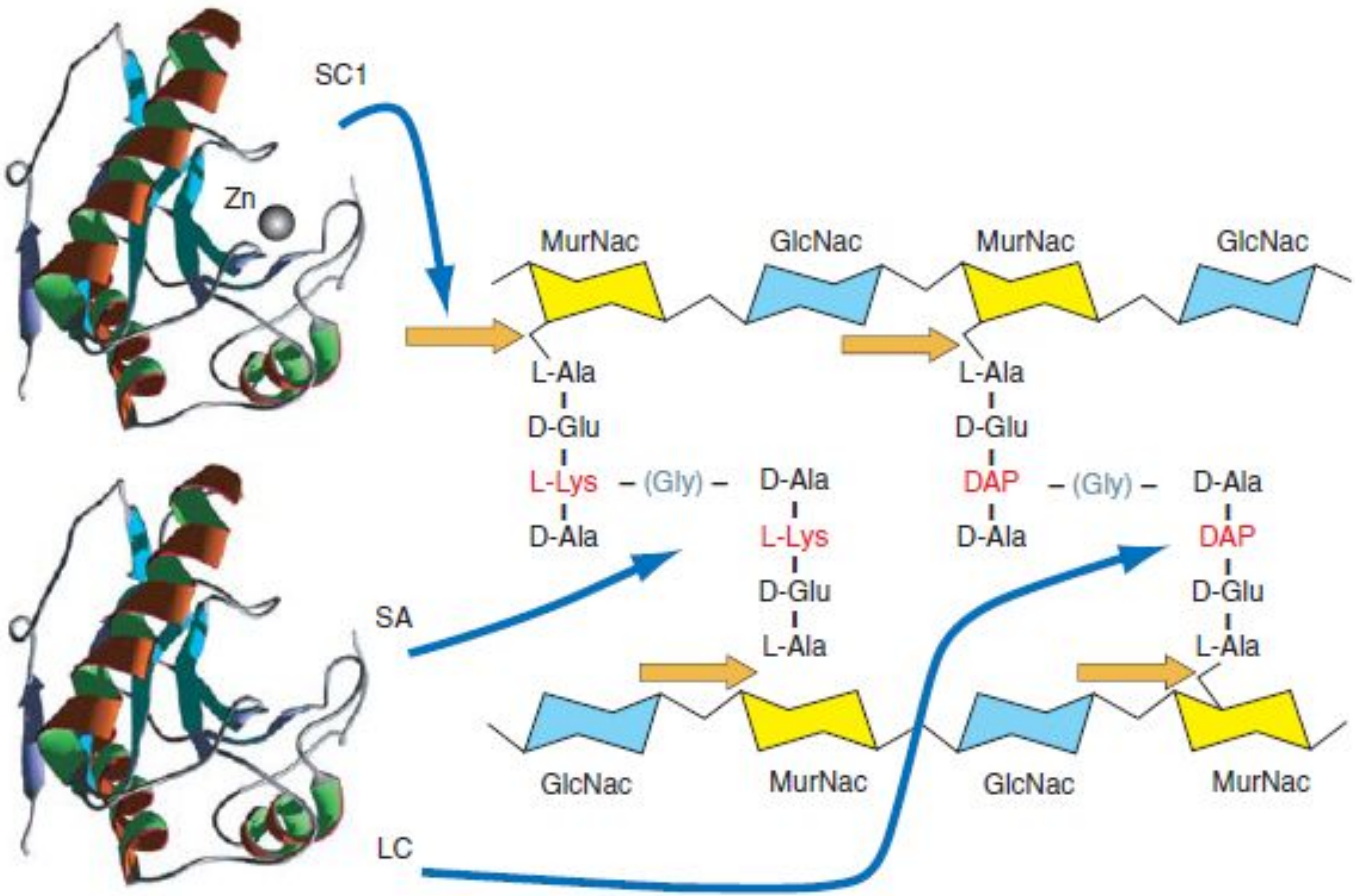


Связывающие пептидогликан белки PGRP дрозофилы

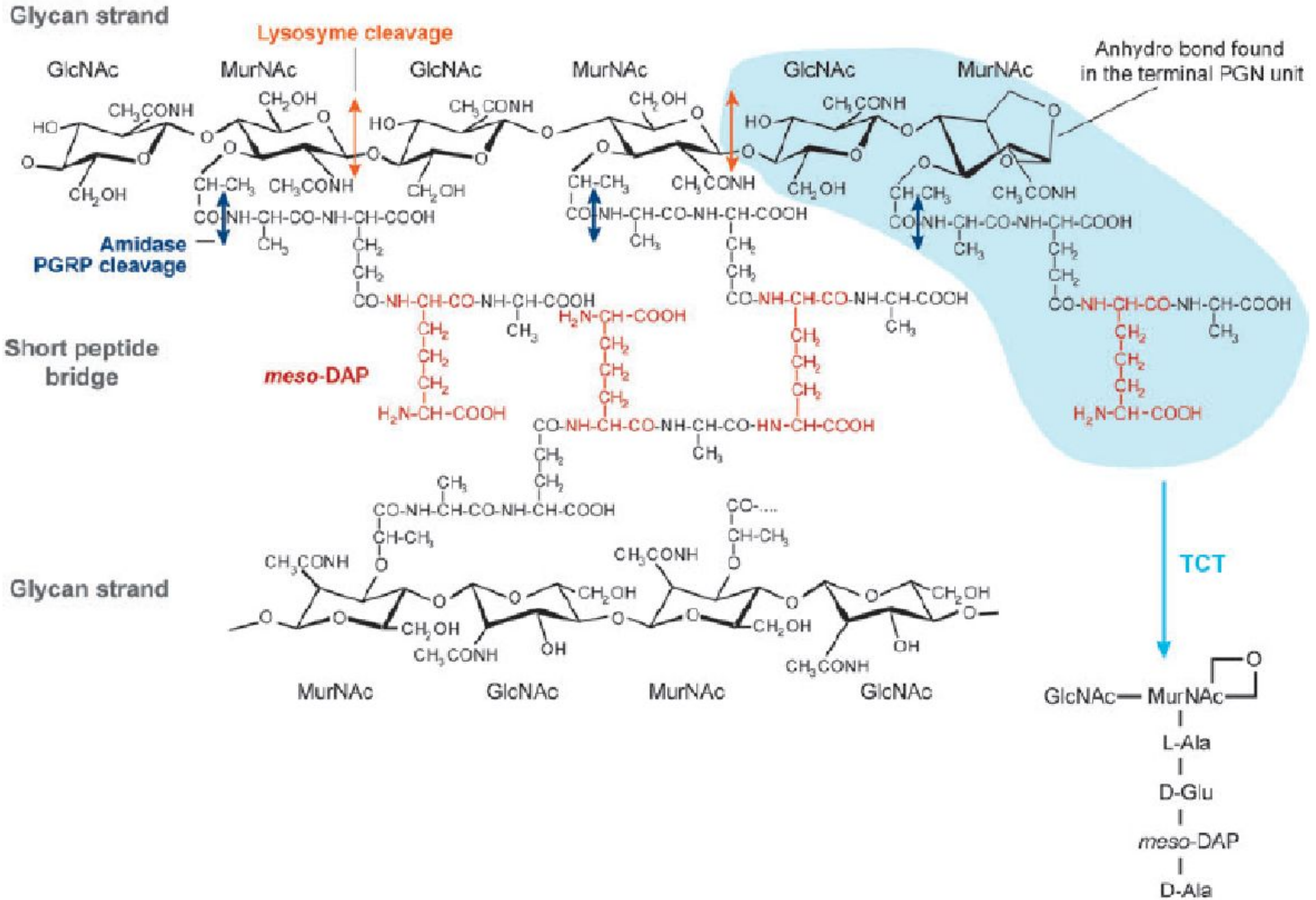
Домен PGRP похож по структуре на лизоцим фага Т7



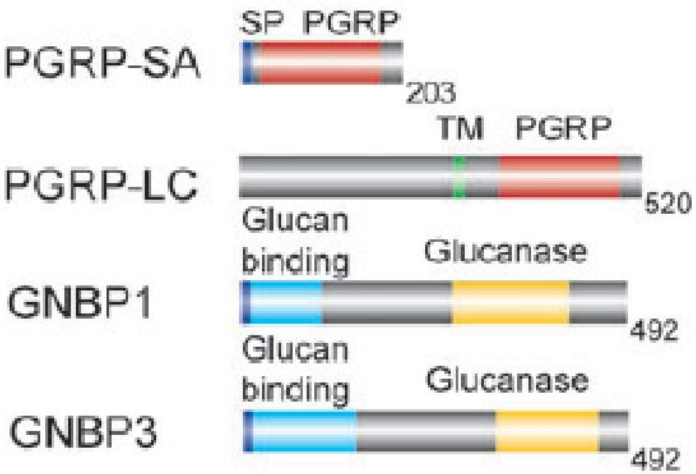
У **части PGRP активный центр уже не расщепляет пептидогликан, но специфически распознает его.**

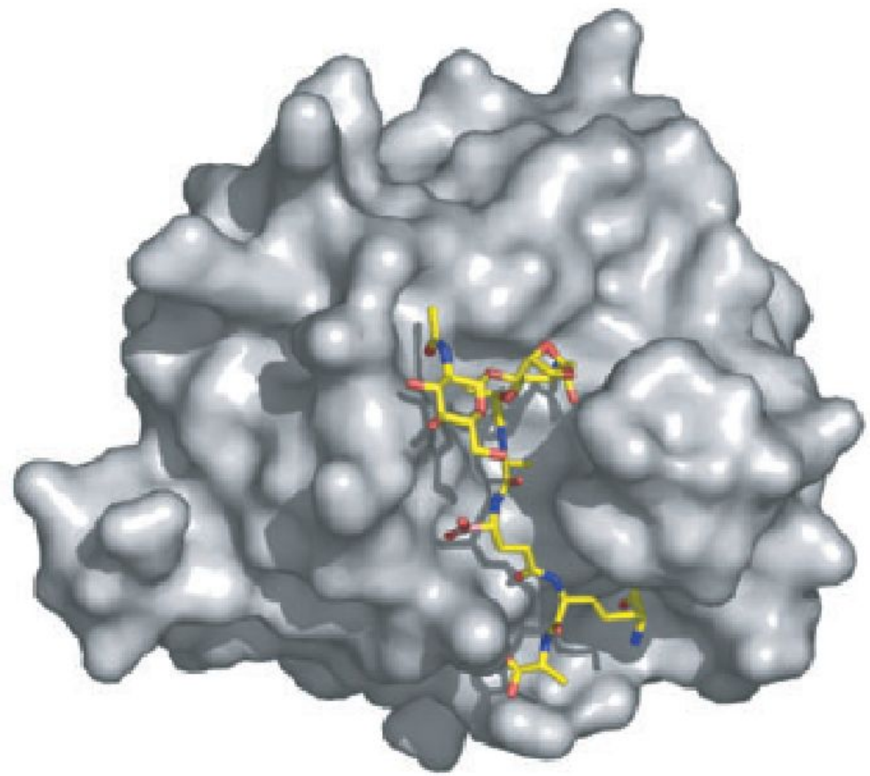


Продукты расщепления пептидогликана



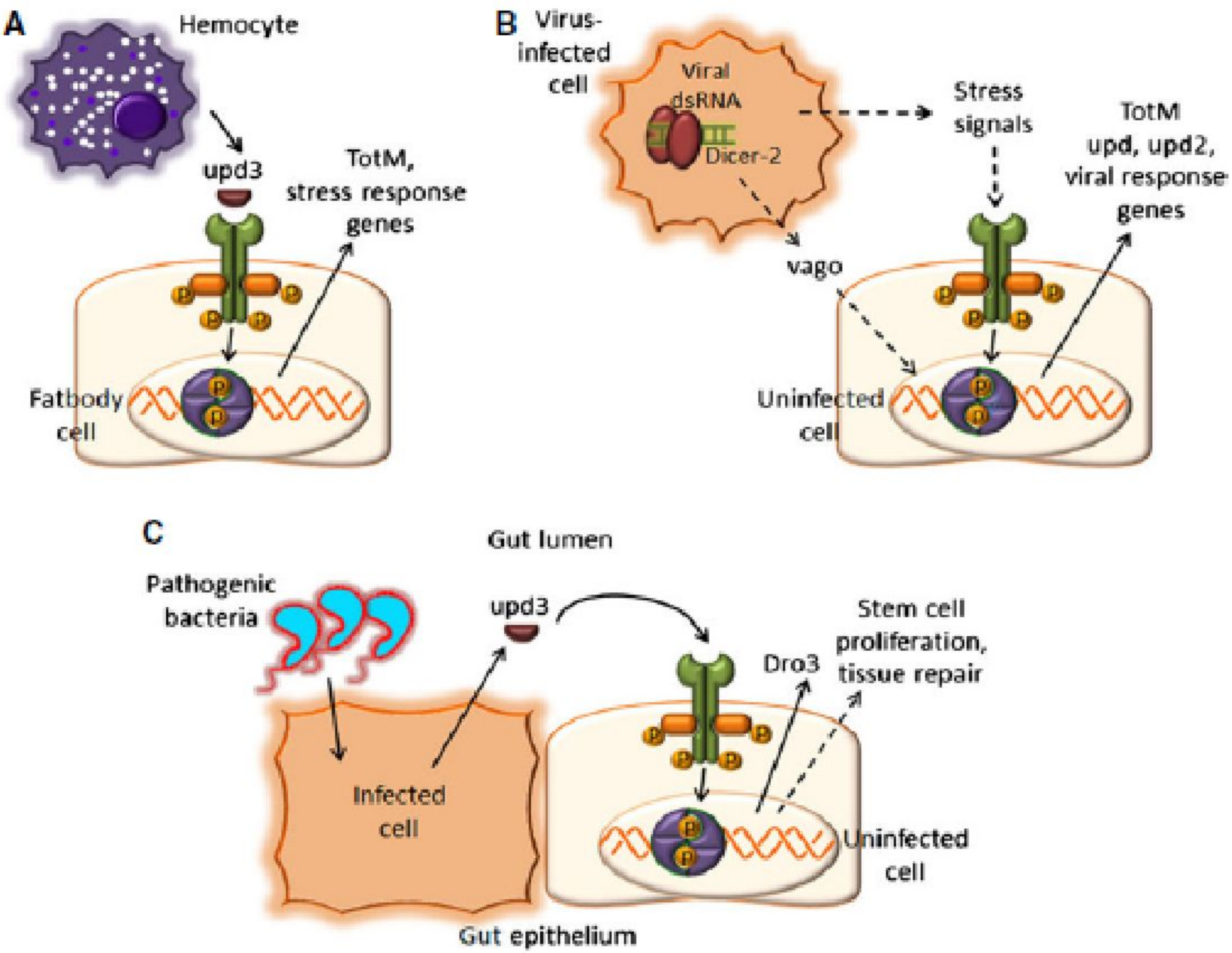
Белки PGRP и NGBP PGRP-LE связавшийся с TCT



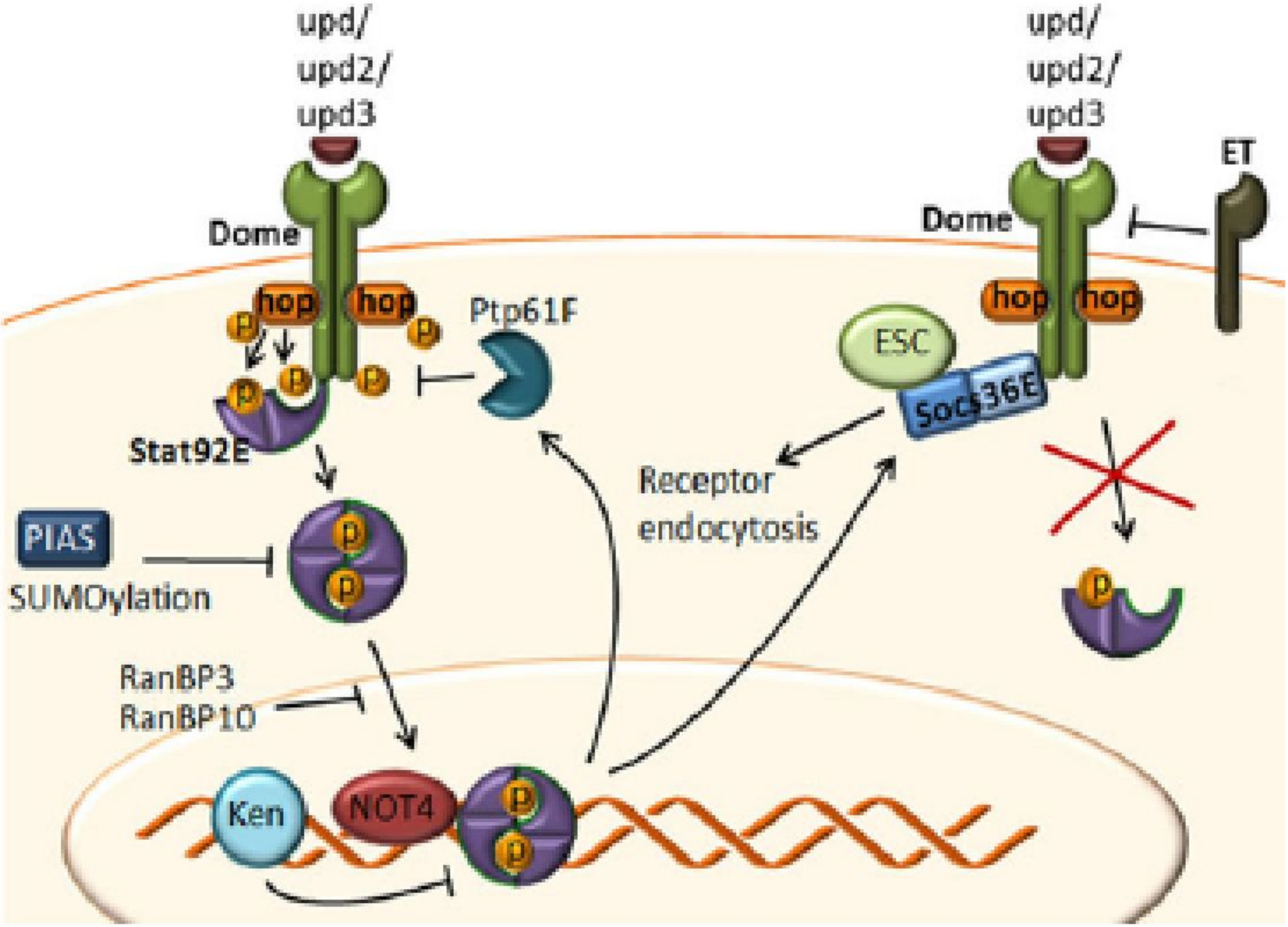


TCT – трахеальный цитотоксин, фрагмент пептидогликана с формулой GlcNAc-MurNAc (anhydro)-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala

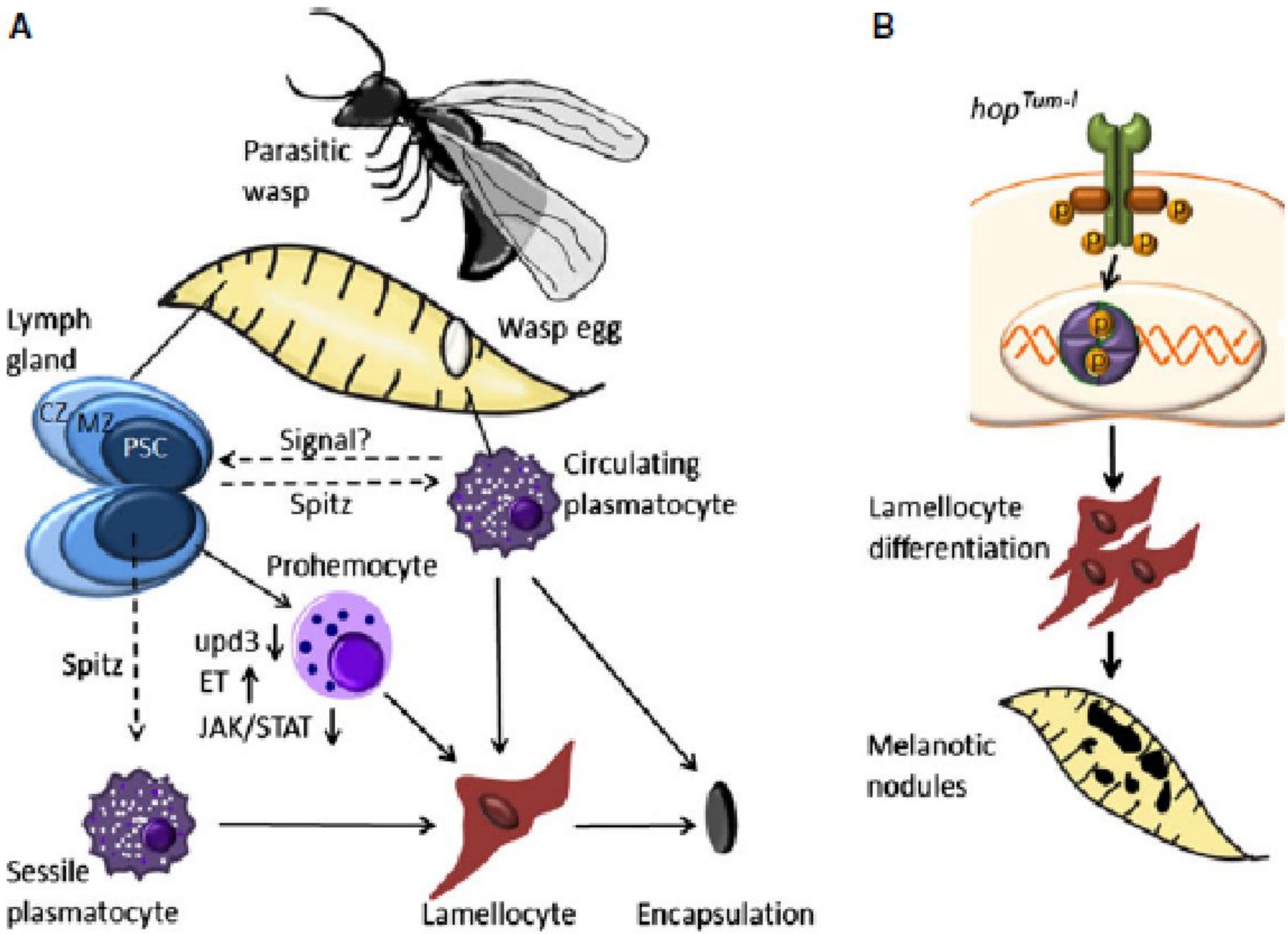
У взрослой мухи JAK/STAT нужен для запуска системного иммунного ответа жировым телом, противовирусного ответа и регуляции регенерации.

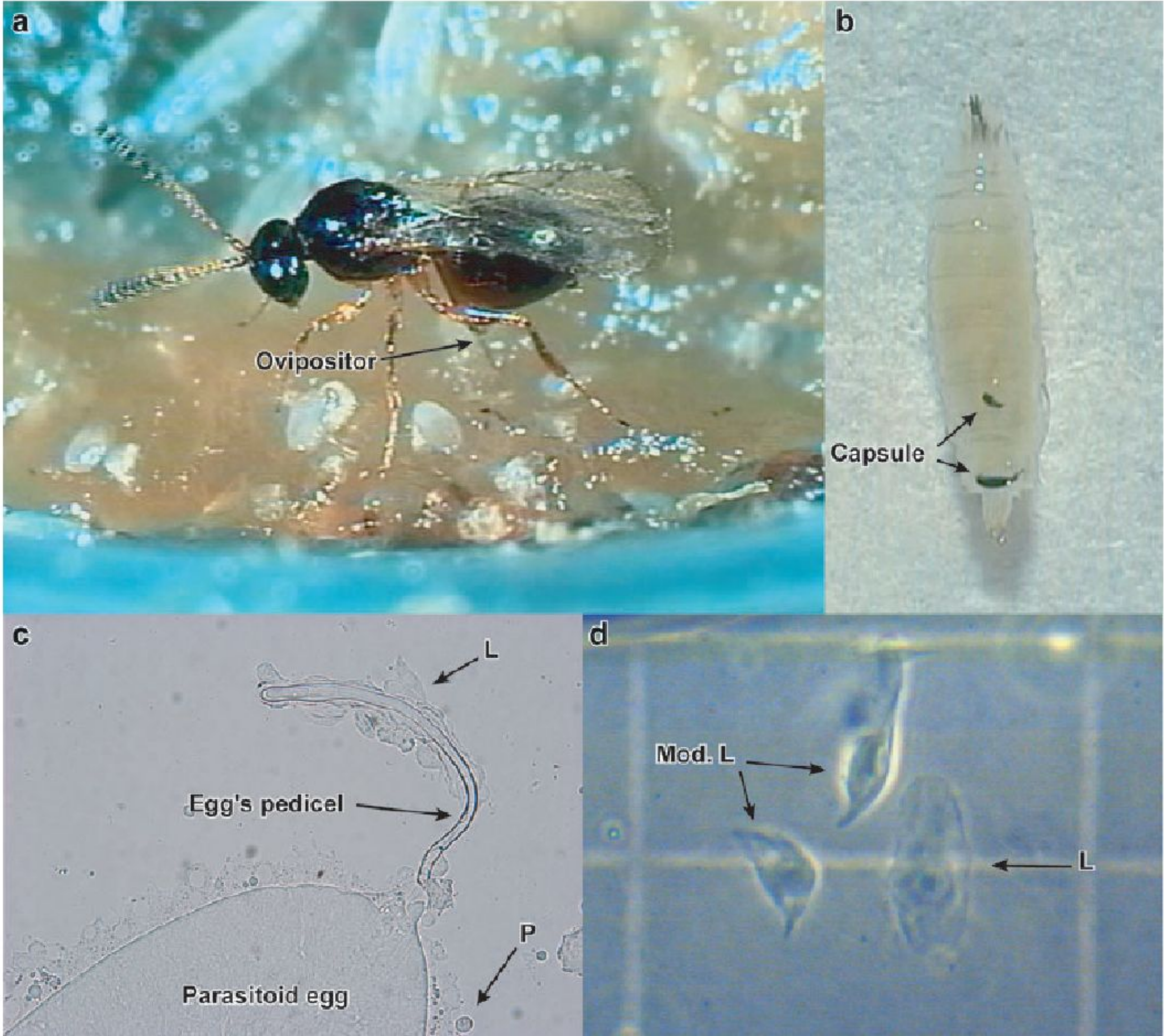


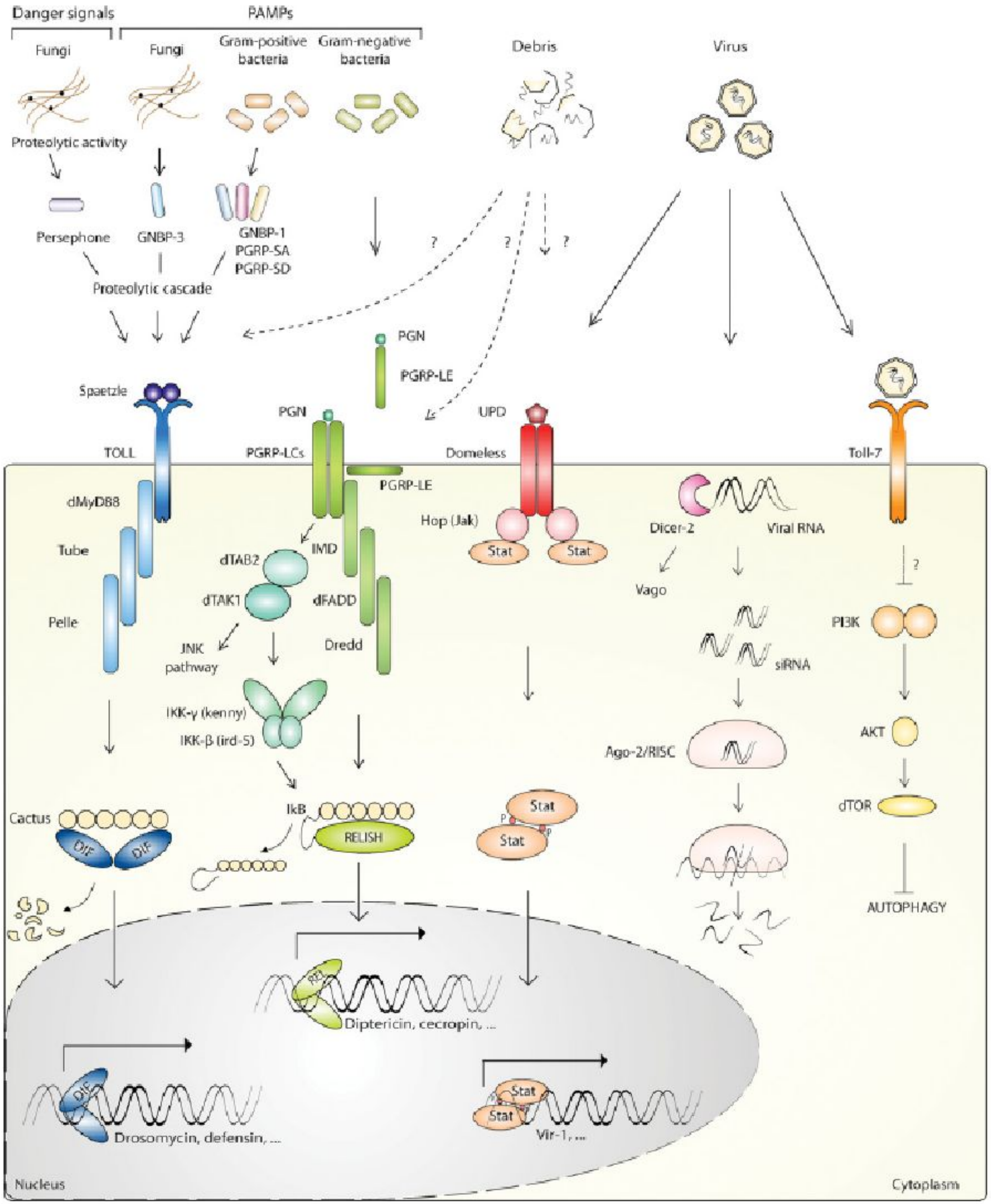
JAK/STAT путь



JAK/STAT путь регулирует инкапсуляцию, но может приводить к возникновению опухолей

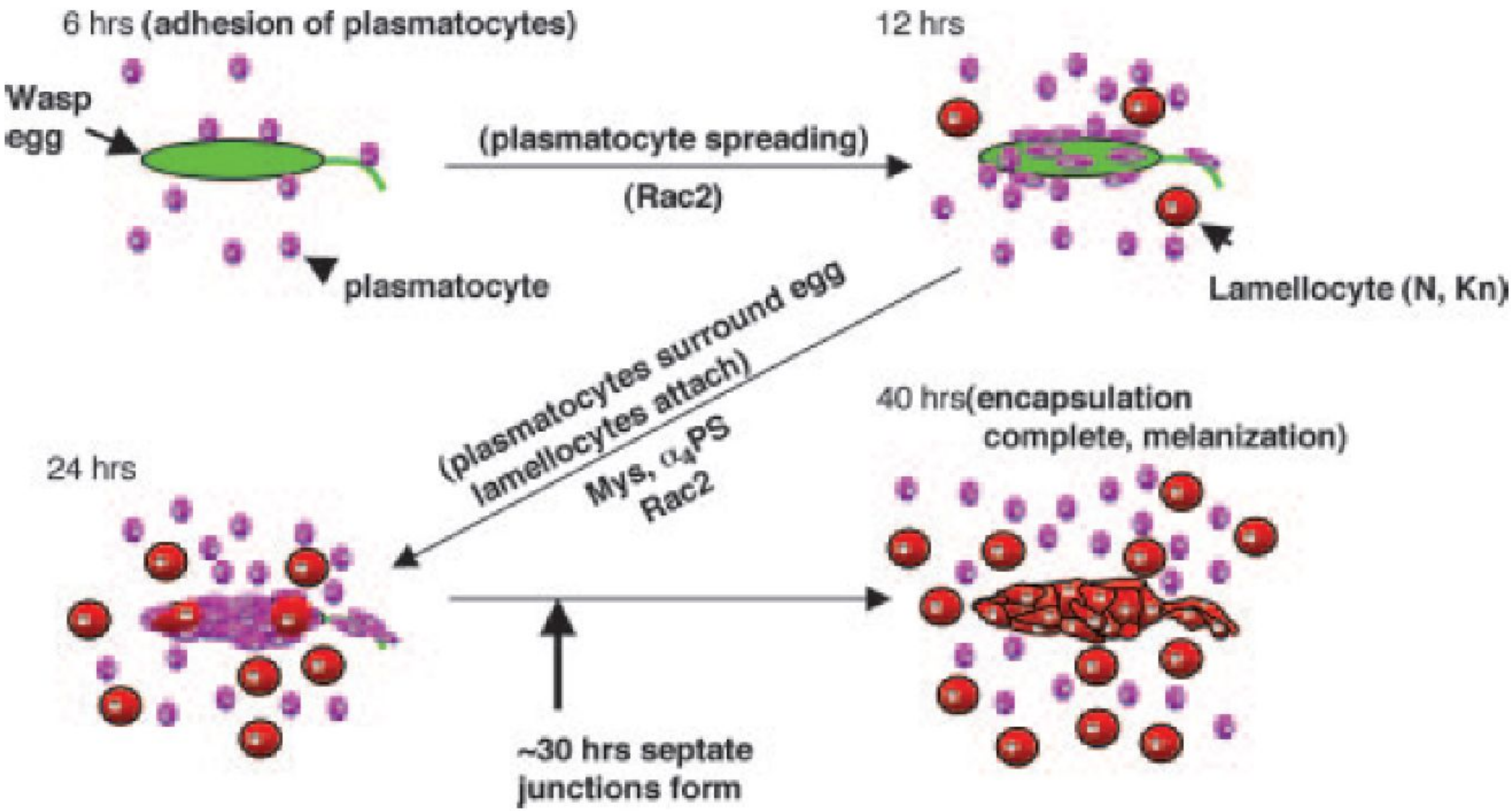



a
Ovipositor
b
Capsule
c
L
Egg's pedicel
P
Parasitoid egg
d
Mod. L
L

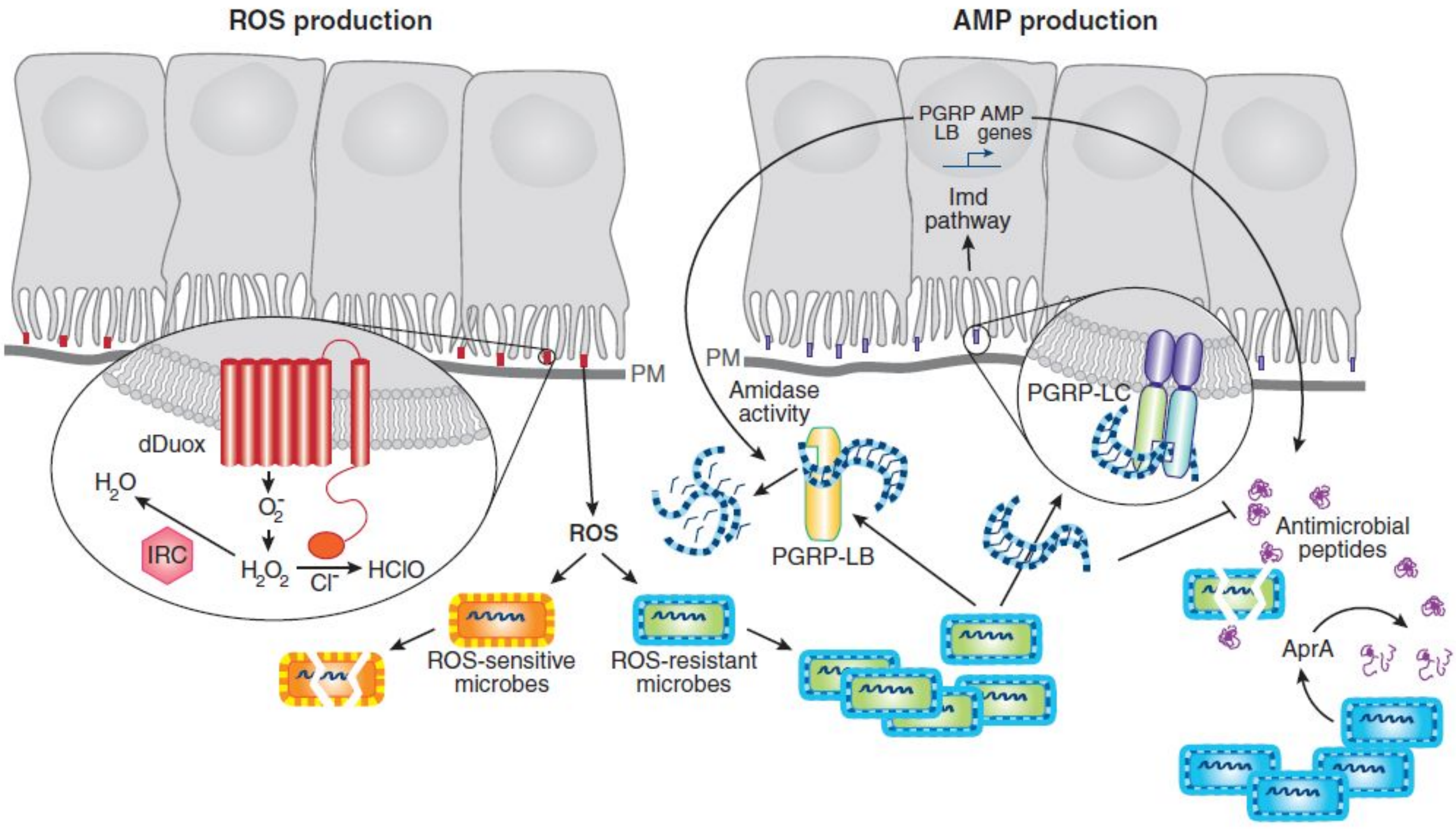


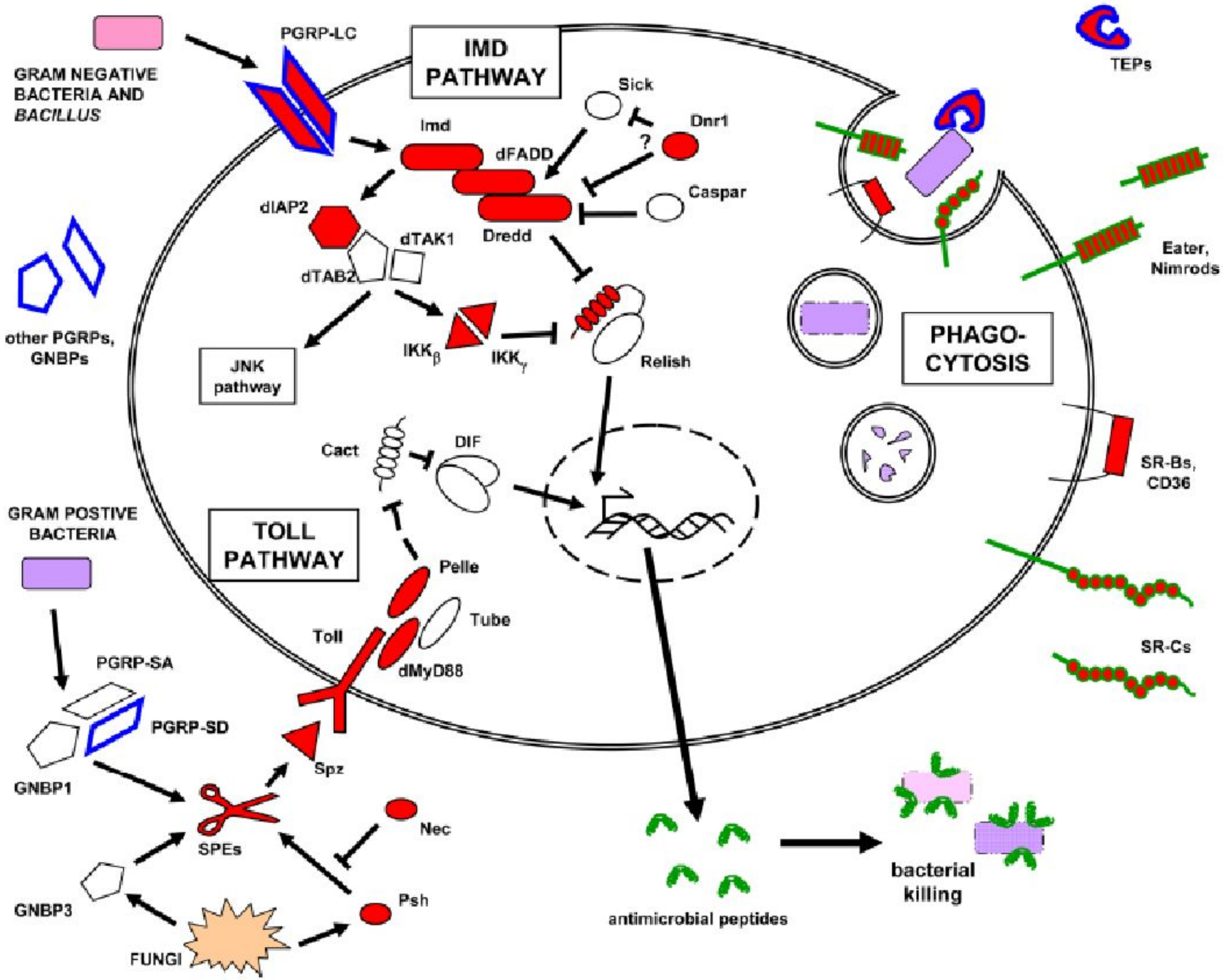
Danger signals
PAMPs
Fungi
Fungi
Gram-positive bacteria
Gram-negative bacteria
Debris
Virus
Proteolytic activity
Persephone
GNBP-3
GNBP-1
PGRP-SA
PGRP-SD
Proteolytic cascade
?
?
?
PGN
PGRP-LE
Spaetzle
PGN
UPD
TOLL
PGRP-LCs
Domeless
Toll-7
PGRP-LE
dMyD88
Tube
Pelle
dTAB2
dTAK1
IMD
dFADD
Dredd
JNK pathway
IKK-γ (kenny)
IKK-β (ird-5)
Hop (Jak)
Stat
Stat
Dicer-2
Viral RNA
Vago
siRNA
Ago-2/RISC
?
PI3K
AKT
dTOR
AUTOPHAGY
Cactus
DIF
DIF
IkB
RELISH
Stat
Stat
REL
Diptericin, cecropin, ...
DIF
Drosomycin, defensin, ...
Stat
Stat
Vir-1, ...
Nucleus
Cytoplasm

Инкапсуляция яйца паразитоида



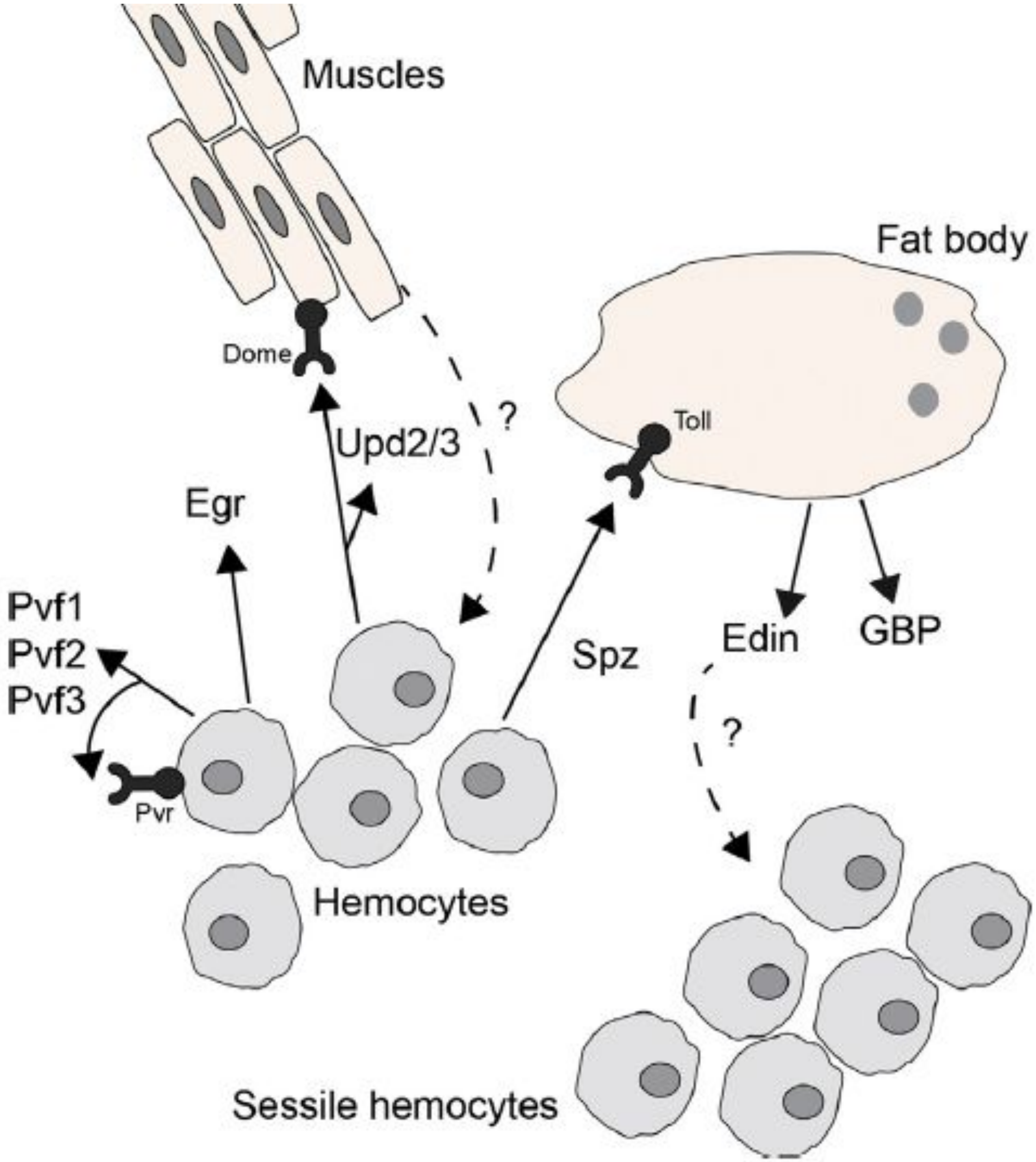
Иммунитет в кишечнике мухи



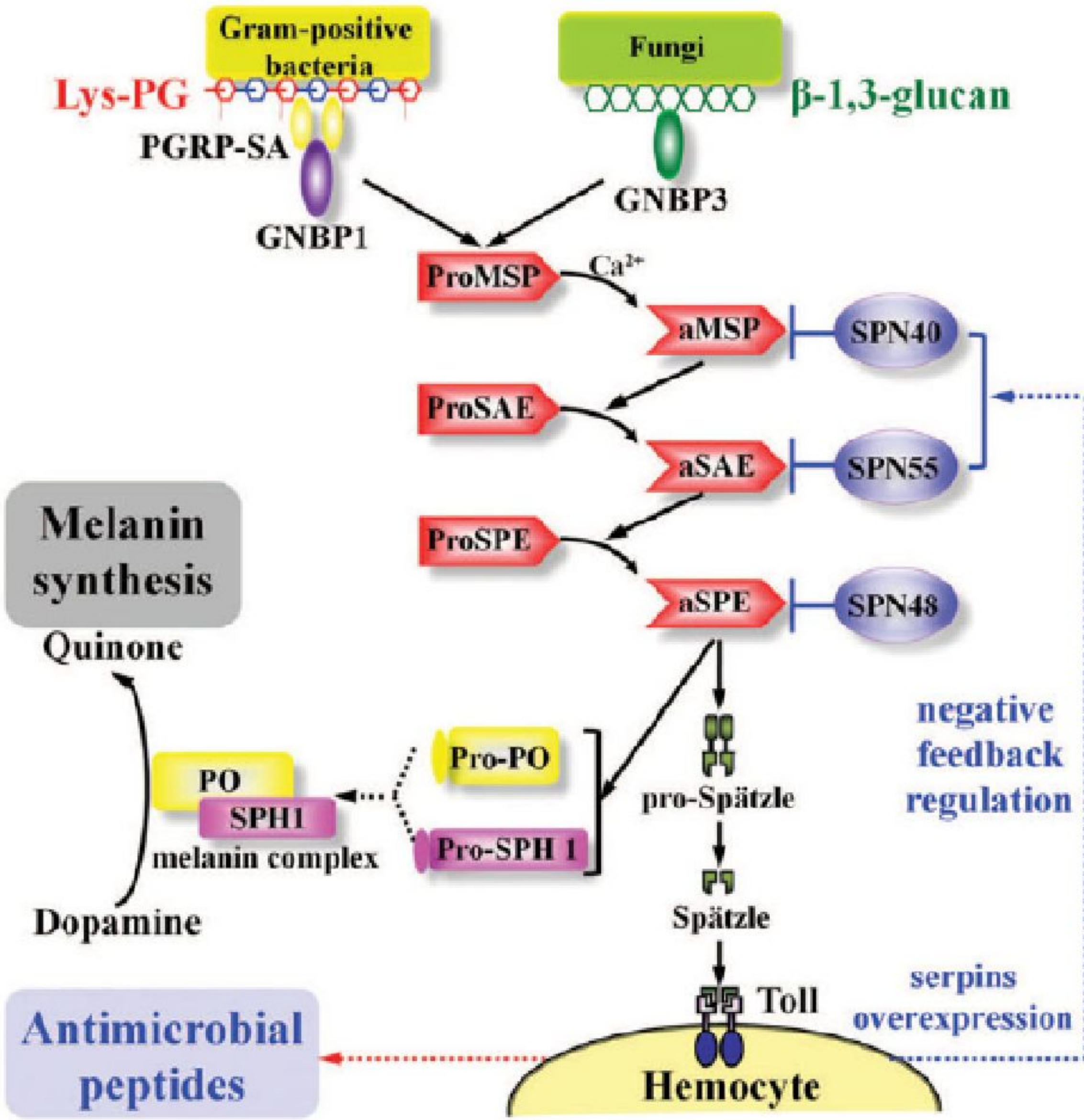


GRAM NEGATIVE BACTERIA AND *BACILLUS*
PGRP-LC
IMD PATHWAY
Sick
Dnr1
?
Imd
dFADD
Caspar
dIAP2
Dredd
dTAK1
dTAB2
other PGRPs, GNBPs
JNK pathway
IKK_β
IKK_γ
Relish
TEPs
Eater, Nimrods
PHAGO-CYTOSIS
SR-Bs, CD36
Cact
DIF
TOLL PATHWAY
GRAM POSTIVE BACTERIA
Pelle
Tube
Toll
dMyD88
PGRP-SA
PGRP-SD
SR-Cs
GNBP1
Spz
SPEs
Nec
GNBP3
Psh
FUNGI
antimicrobial peptides
bacterial killing

Регуляция иммунных реакций дрозофилы



Обратные связи и кросс-токи иммунного ответа идут при участии серпинов



Gene	Type of Protein	Putative Ligand	Evidence
18 wheeler, Toll-2	Toll-like Receptor	Unknown	Expression of Attacin affected in mutant flies.
Toll-9	Toll-like Receptor	Unknown	Protein activates Drosomycin in S2 cells through MyD88.
Ird7, totem, PGRP-LC	Peptidoglycan recognition protein	DAP-type peptidoglycans, G- bacteria	Activate IMD pathway in vivo. Phagocytosis of *E. coli* in S2 cell lines, affected upon RNAi.
PGRP-LE	Peptidoglycan recognition protein	DAP-type peptidoglycans, G- bacteria	Overexpression activates PPO cascade in cell lines. Help PGRP-LC recognize peptidoglycans.
Semmelweis, PGRP-SA	Peptidoglycan recognition protein	Lys-type peptidoglycans, G+ bacteria	Activation of Toll and phagocytosis of *S. aureus* pathway affected in mutants.
PGRP-SD	Peptidoglycan recognition protein	Lys-type peptidoglycans G+ bacteria	Activation of Toll pathway affected in double mutants with PGRP-SA mutants.
Picky, PGRP-SC1a	Peptidoglycan recognition protein	G+ bacterial peptidoglycans	Activation of Toll pathway and *S. aureus* phagocytosis affected in mutants.
PGRP-SC1b	Peptidoglycan recognition protein	G+ bacterial peptidoglycans	Cleaves *S. aureus* peptidoglycans.
Osiris, GNBP1	Gram-negative binding protein	Potentially G+ bacterial determinants	Hydrolyzes G+ peptidoglycan. Acts in complex with PGRP-SA to activate Toll pathway.
TEPs	Thiolester containing proteins	Possibly binding to bacterial surface	RNAi of homologous mosquito gene reduces phagocytosis of Gram-negative bacteria. Plasmodia population larger in *Tep1* mutant mosquito, causing higher vectorial capacity. RNAi of Tep2 decreases uptake of *E. coli*; RNAi of Tep3 decreases uptake of *S. aureus*.
Mcr	Macroglobulin complement-related	*Candida albicans*	RNAi of S2 cells reduces phagocytosis of *C. albicans*
dSR-C1	Scavenger receptor	Possibly both G+ and G- bacteria	RNAi of S2 cells reduces phagocytosis of bacteria.
Crq (Croquemort)	Scavenger receptor, CD36 like	Apoptotic cells and possibly G+ bacteria	Phagocytosis of *S. aureus* impaired in cell lines.
Peste	Scavenger receptor, CD36 like	Mycobacteria	RNAi of S2 cells reduces phagocytosis of Mycobacteria.
Eater	Scavenger receptor, epidermal growth factor like	Possibly G+ and G- bacteria	Reduction in phagocytosis of *S. aureus* and *E. coli* in vitro in cell-line mutants and in vivo in deficiency. Increased susceptibility to natural infection in vivo.

- Следующая лекция 12 марта – снова об иммунной системе дрозофилы