

# Эволюционная иммунология

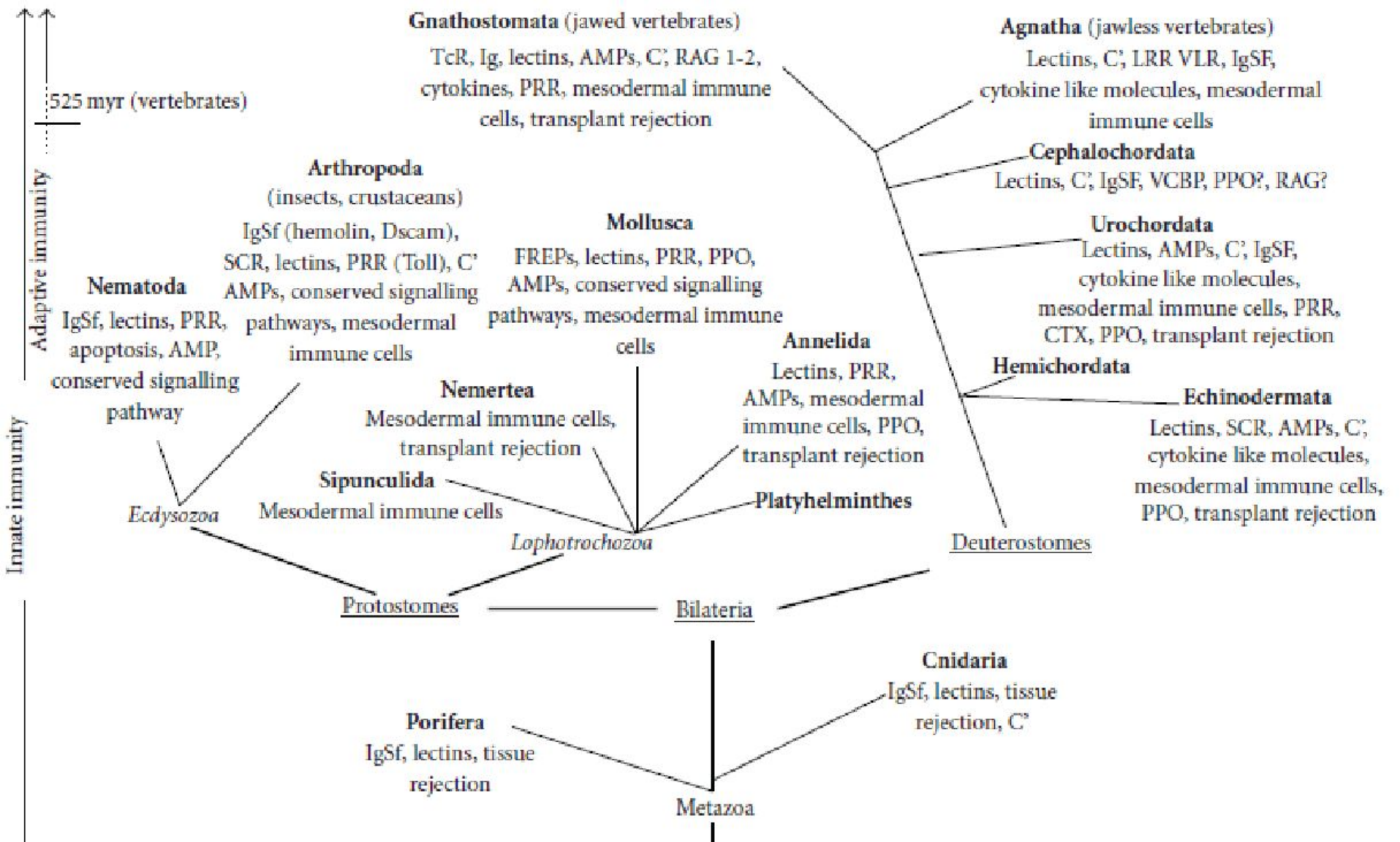
## Лекция 6 «Разнообразии иммунных реакций беспозвоночных»



Шилов Е.С.  
19 марта 2018

# Иммуноциты беспозвоночных

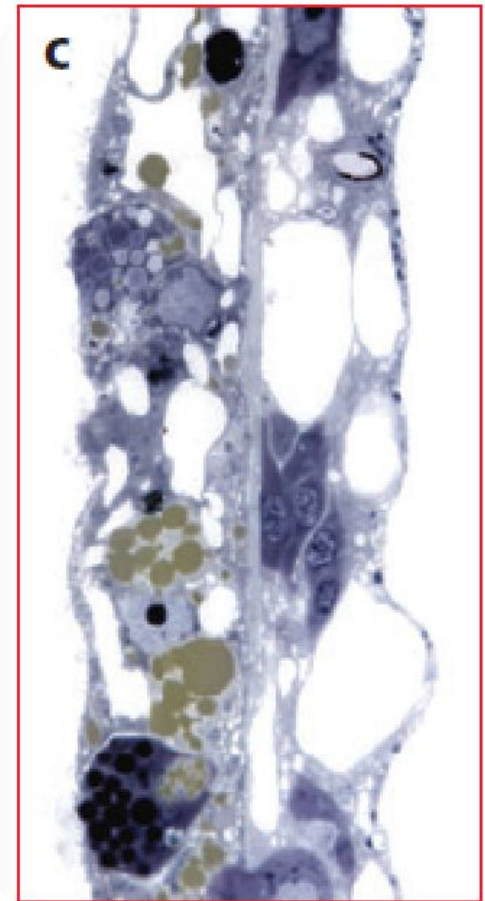
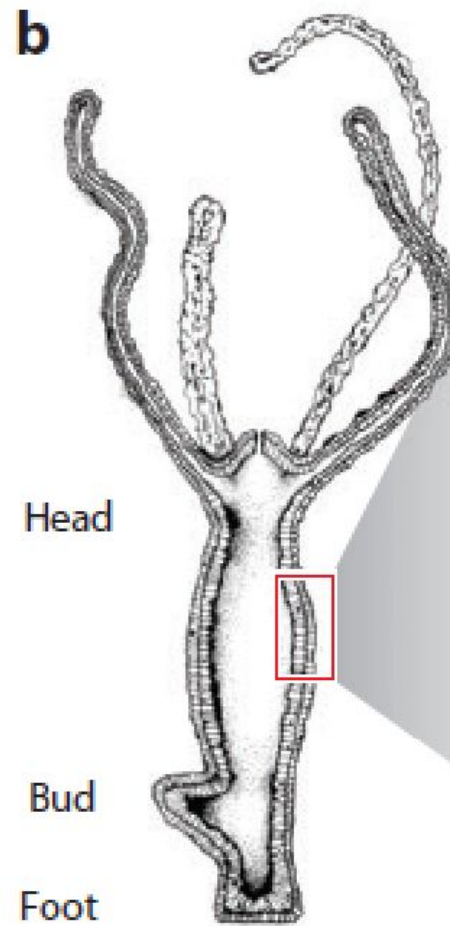
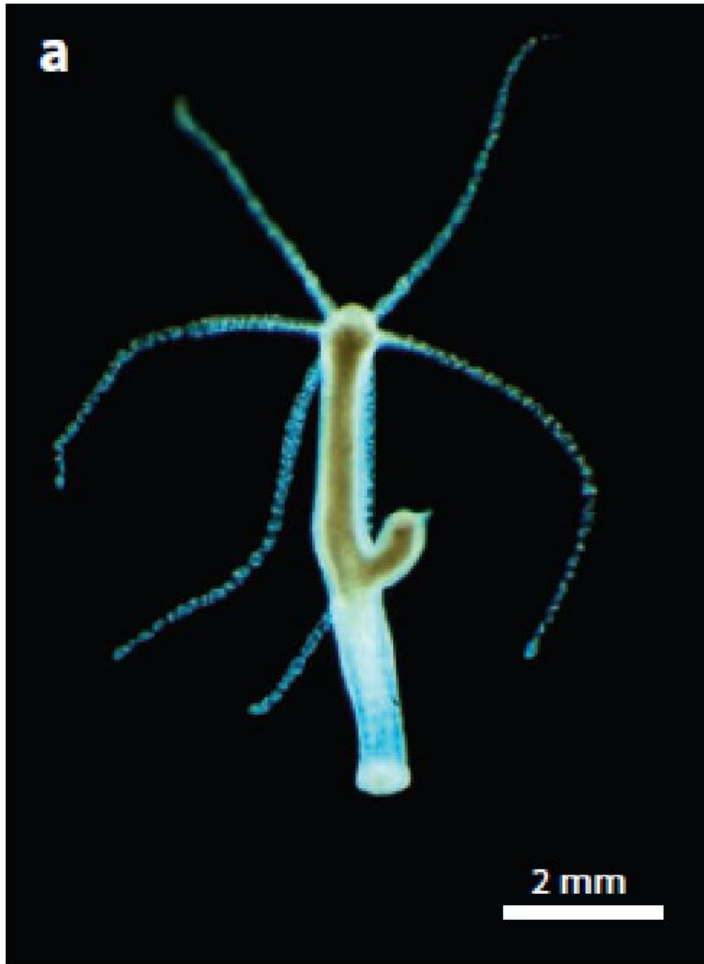
Таксон	Макрофагоподобные клетки	Лимфоциты и лимфоцитоподобные клетки	Очаги гемопоэза
Губки	Блуждающие амебоциты (архециты)	—	—
Кишечно-полостные	Блуждающие амебоциты	—	—
Немертины	Гиалиновые амебоциты	Лимфоцитоподобные амебоциты	—
Кольчатые черви	Гиалиновые амебоциты (нейтрофилы)	Лимфоцитоподобные клетки двух типов (базофилы)	Парные узелки в целоме, гемопоэтическая ткань в алиментарном кровяном синусе
Моллюски	Гиалиновые амебоциты	Лимфоцитоподобные клетки	Гемопоэтическая ткань целома. «Белое тело» головоногих моллюсков
Членистоногие	Плазматоциты	Прогемоциты	Гемопоэтические участки в различных частях тела
Иглокожие	Фагоцитирующие амебоциты	Лимфоциты	Аксиальный орган
Оболочники	Макрофаги	Лимфоциты	Лимфатические узелки в глоточной стенке, вокруг пищеварительного тракта, в стенках тела



AMP: antimicrobial peptides  
 C': complement pathway elements  
 CTX: cortical thymocytmarker in *Xenopus*  
 Dscam: drosophila homolog for Down syndrome cell adhesion molecule  
 FREPs: fibrinogen related peptides  
 IgSf: immunoglobulin superfamily

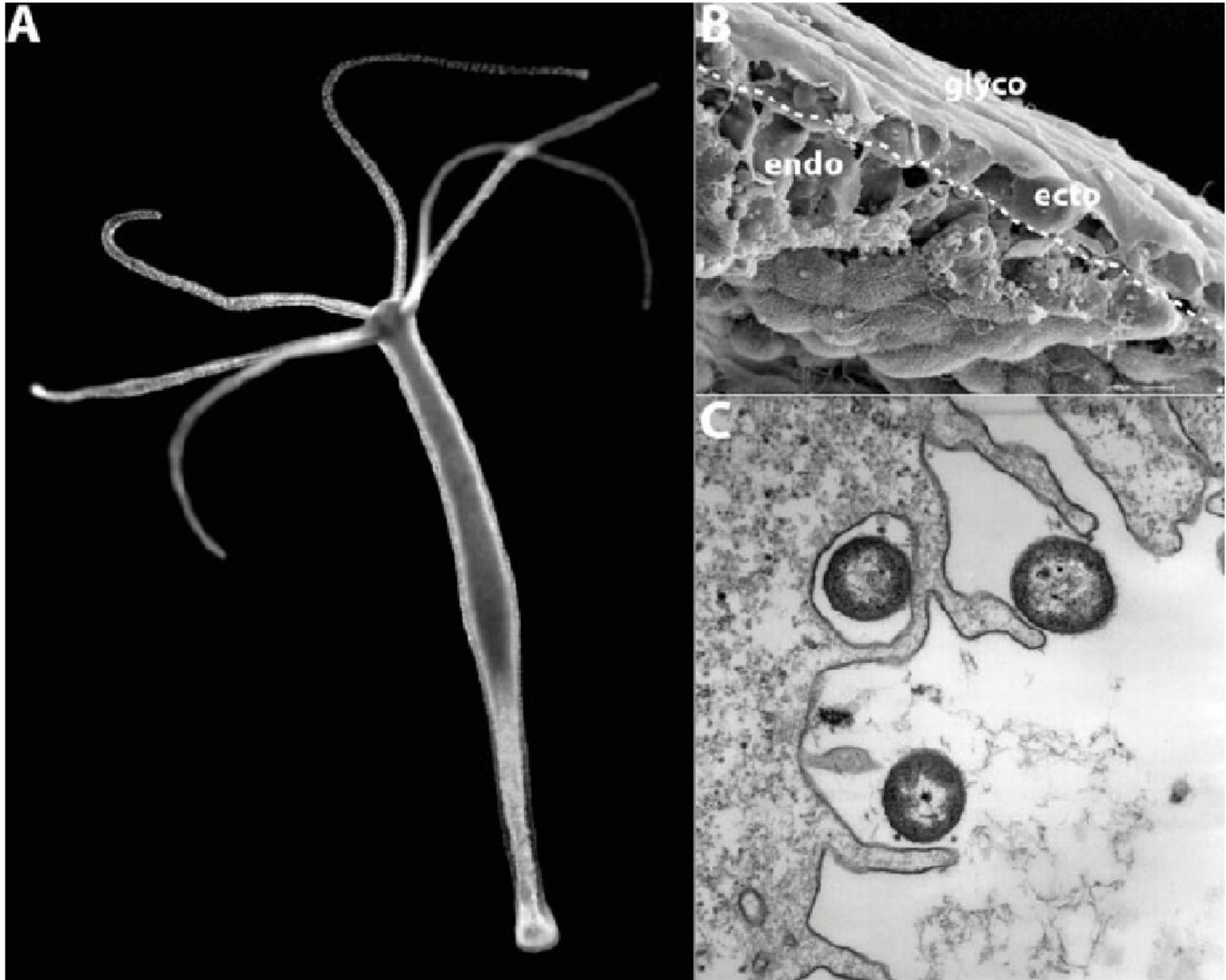
LRR VLR: variable lymphocyte receptors with leucine rich repeats  
 PPO: prophenoloxidase cascade  
 PRR: pattern recognition receptors  
 RAG: recombination-activating genes  
 SCR: scavenger receptors  
 VCBP: variable chitin binding proteins  
 TCR: T cell receptor

# Строение гидры

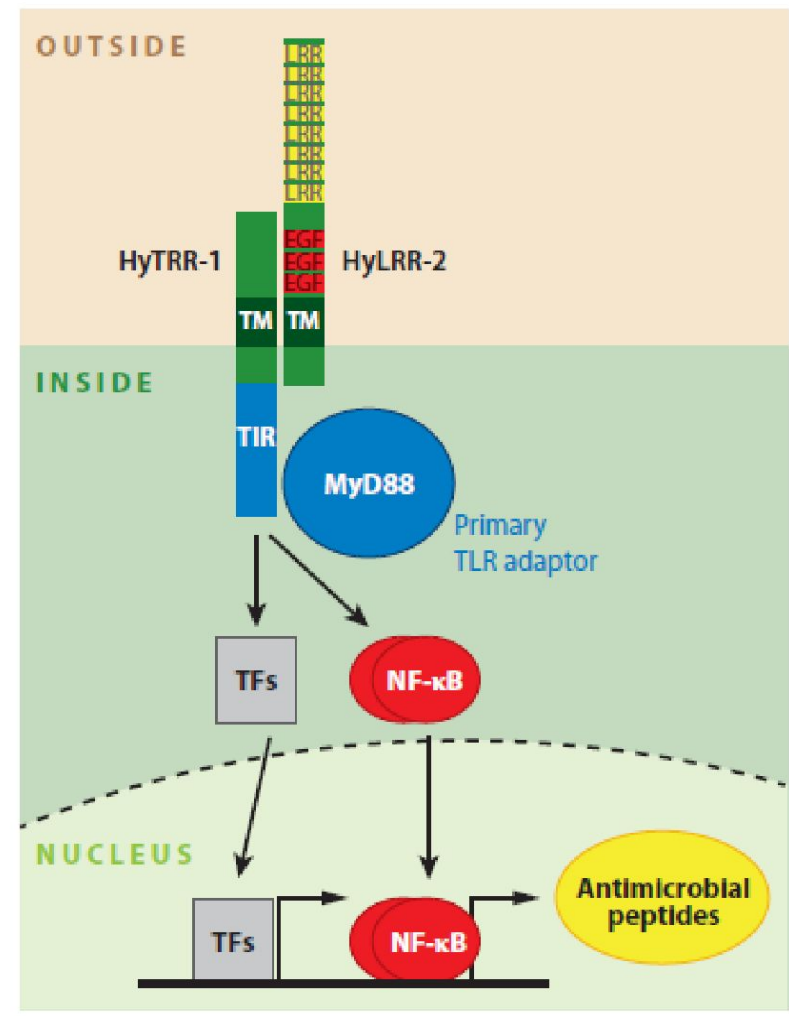
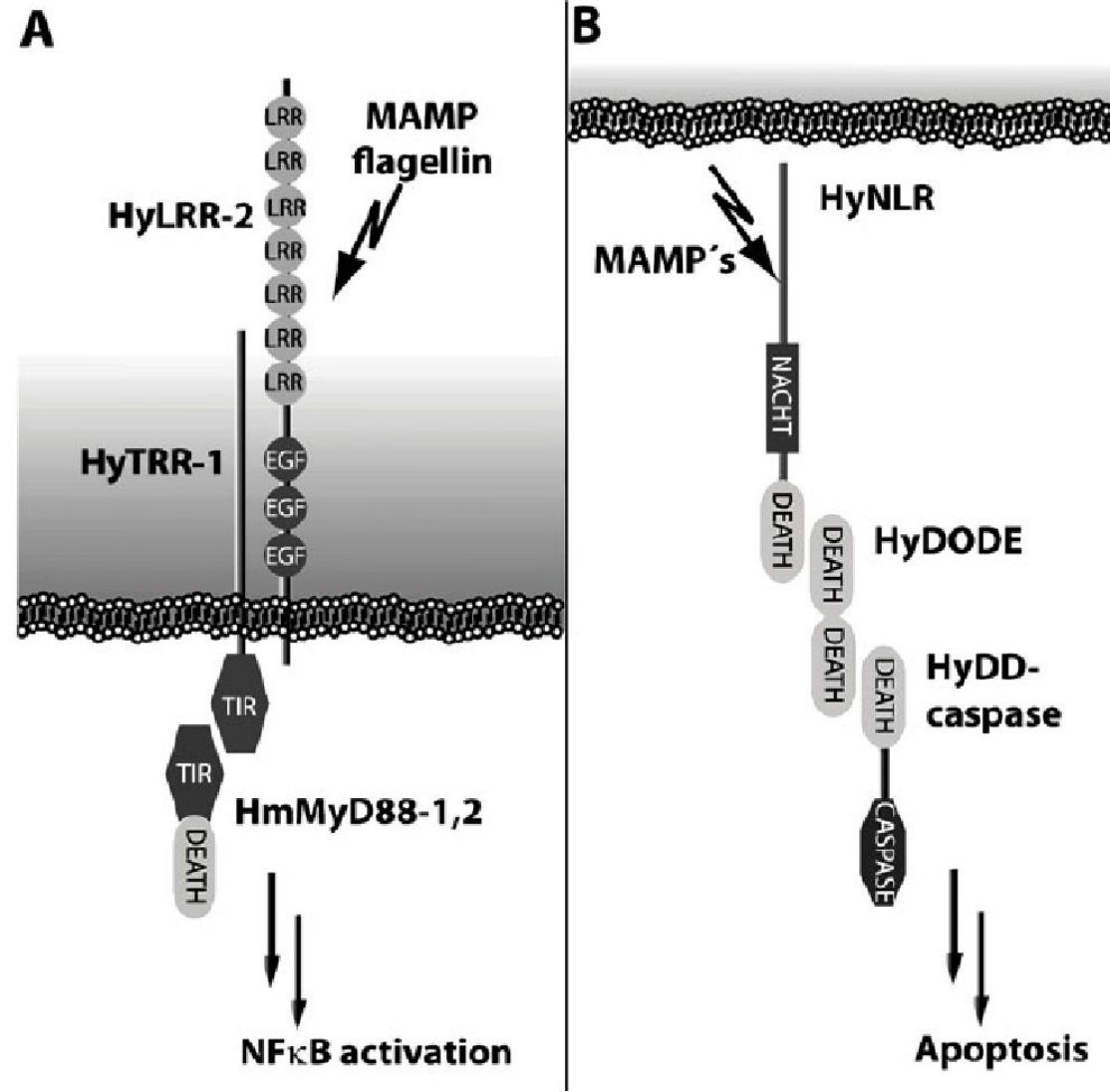


Endoderm ECM Ectoderm  
(mesoglea)

# Эпителиальные клетки гидроидных способны к фагоцитозу



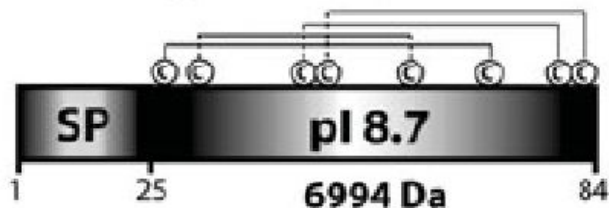
# У кишечнополосчатых есть собственные TLR- и NLR - рецепторы



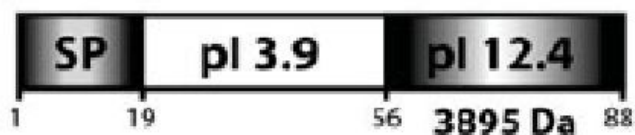
# Антимикробные пептиды книдарий

A

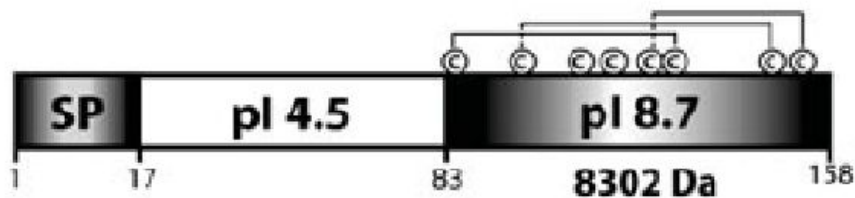
Hydramacin-1



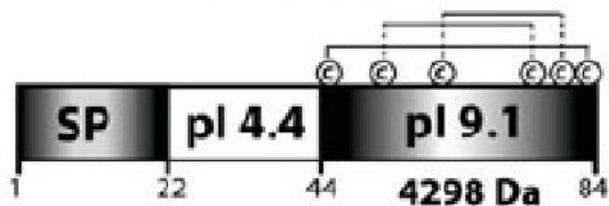
Arminin 1a



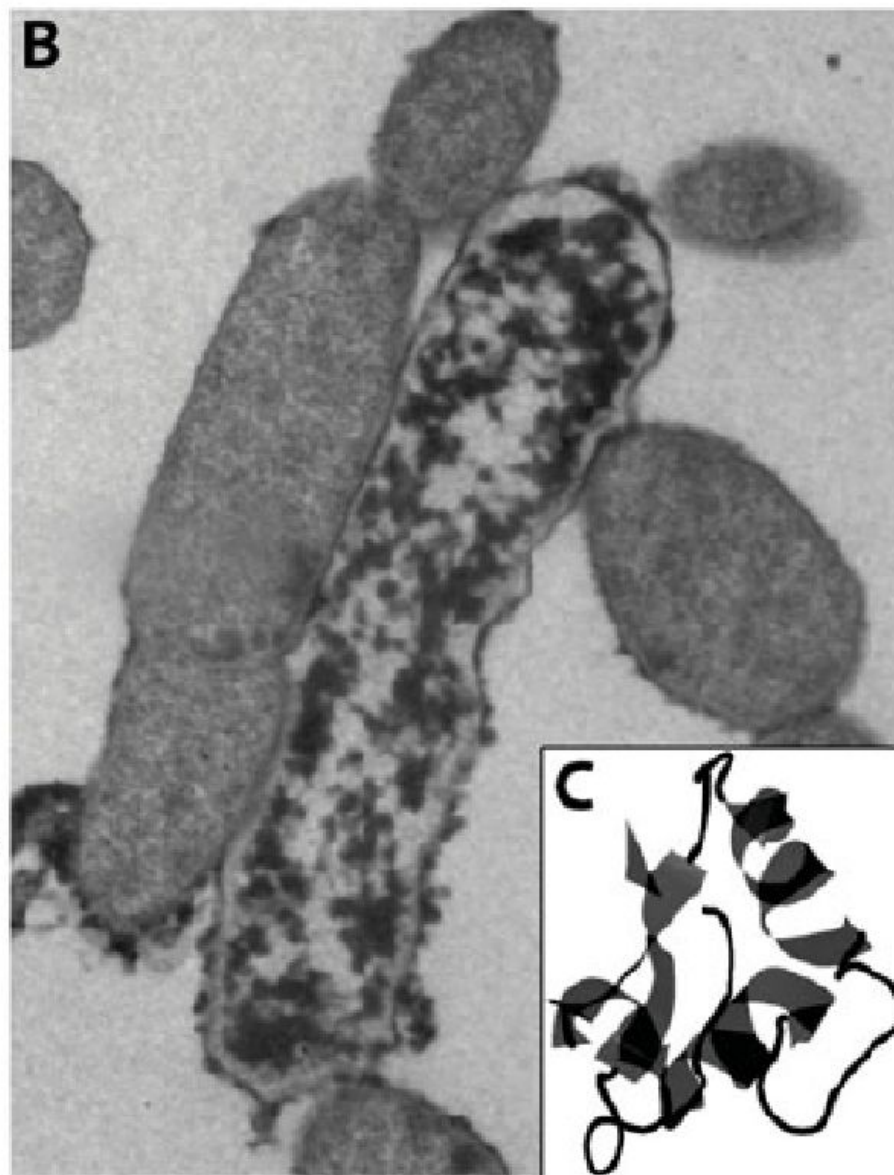
Periculin-1



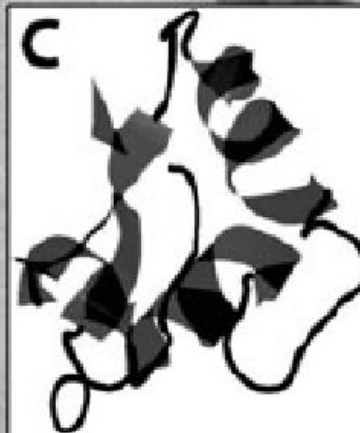
Aurelin



B



C



# Странный белок дождевого червя CCF

Белок жидкости целомической полости массой 42 кДа, распознаёт (?) О-антиген ЛПС, мурамилдипептиды, мурамовую кислоту, бета-люканы, диацетилхитобиозу.

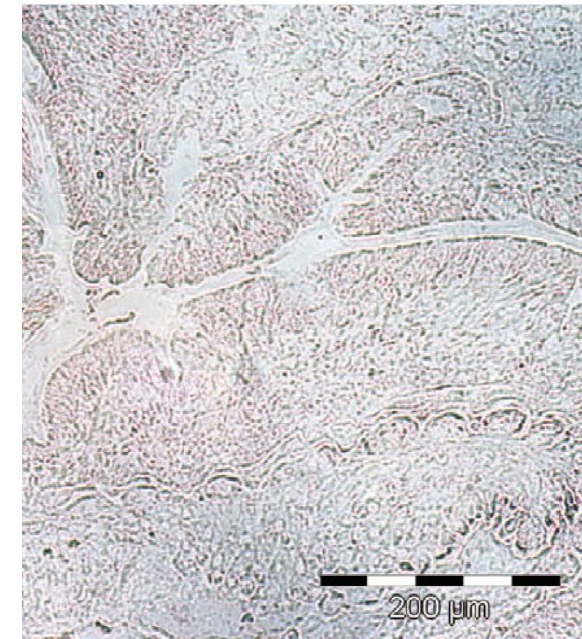
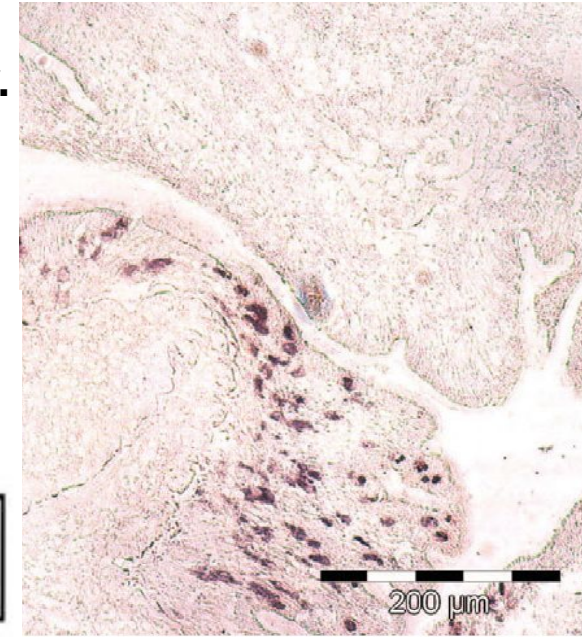
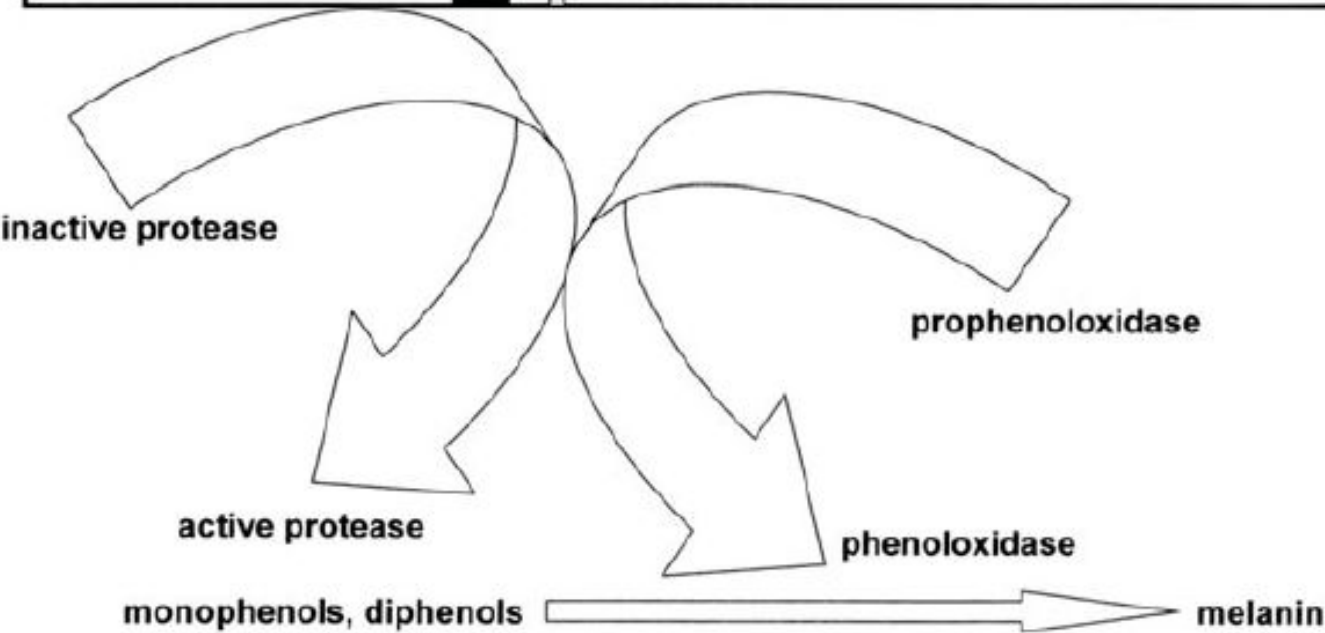
Активирует профенолоксидазу, опсонизирует клетки патогенов, его синтез индуцируется ЛПСом, а сам он лизирует клетки L929 (и другие тоже). Так ли это?

Interaction with  
Gram-negative  
bacteria and  
yeast

Interaction with Gram-positive bacteria,  
trypanosomes and mammalian cells

CCF

W-rich C-terminal part

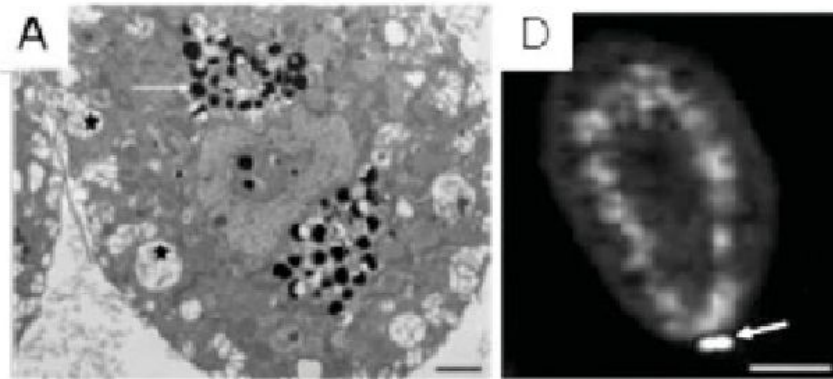




# Хлорогенные клетки, малые целомоциты и амёбоциты кольчатых червей

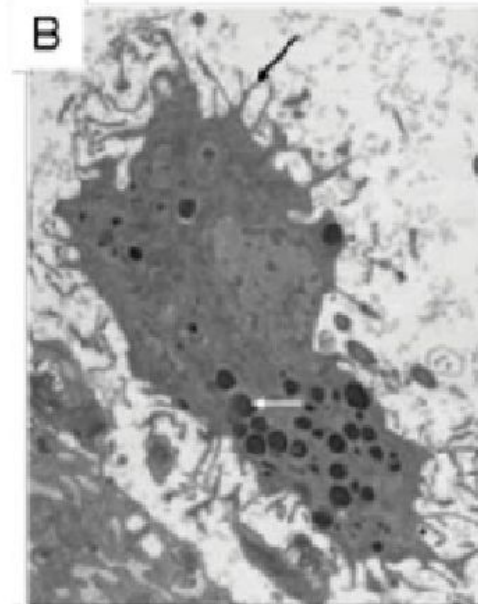
## Chloragocytes (100-150 $\mu\text{m}$ )

- Tt cathepsin L and cystatin B immunoreactivity
- Bacteria binding



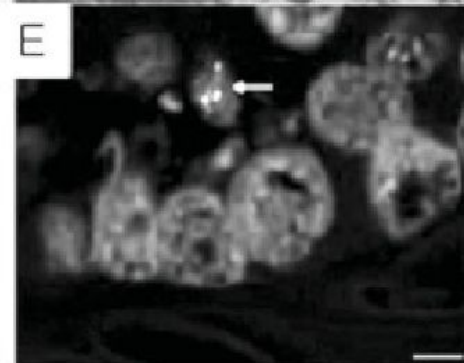
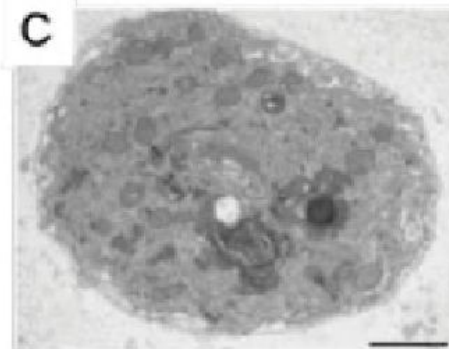
## Granular amoebocytes (30-70 $\mu\text{m}$ )

- Tt cathepsin L immunoreactivity
- Migrate to the infection site
- Phagocyte Gram + and - bacteria



## Small coelomocytes (7-12 $\mu\text{m}$ )

- peptide B production



# Антимикробные пептиды пиявки

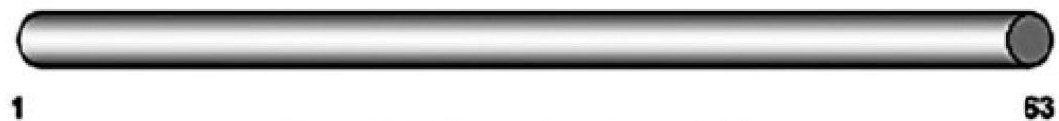
Theromacin, **cationic**, anti Gram +



Theromyzin, **anionic**, anti Gram +



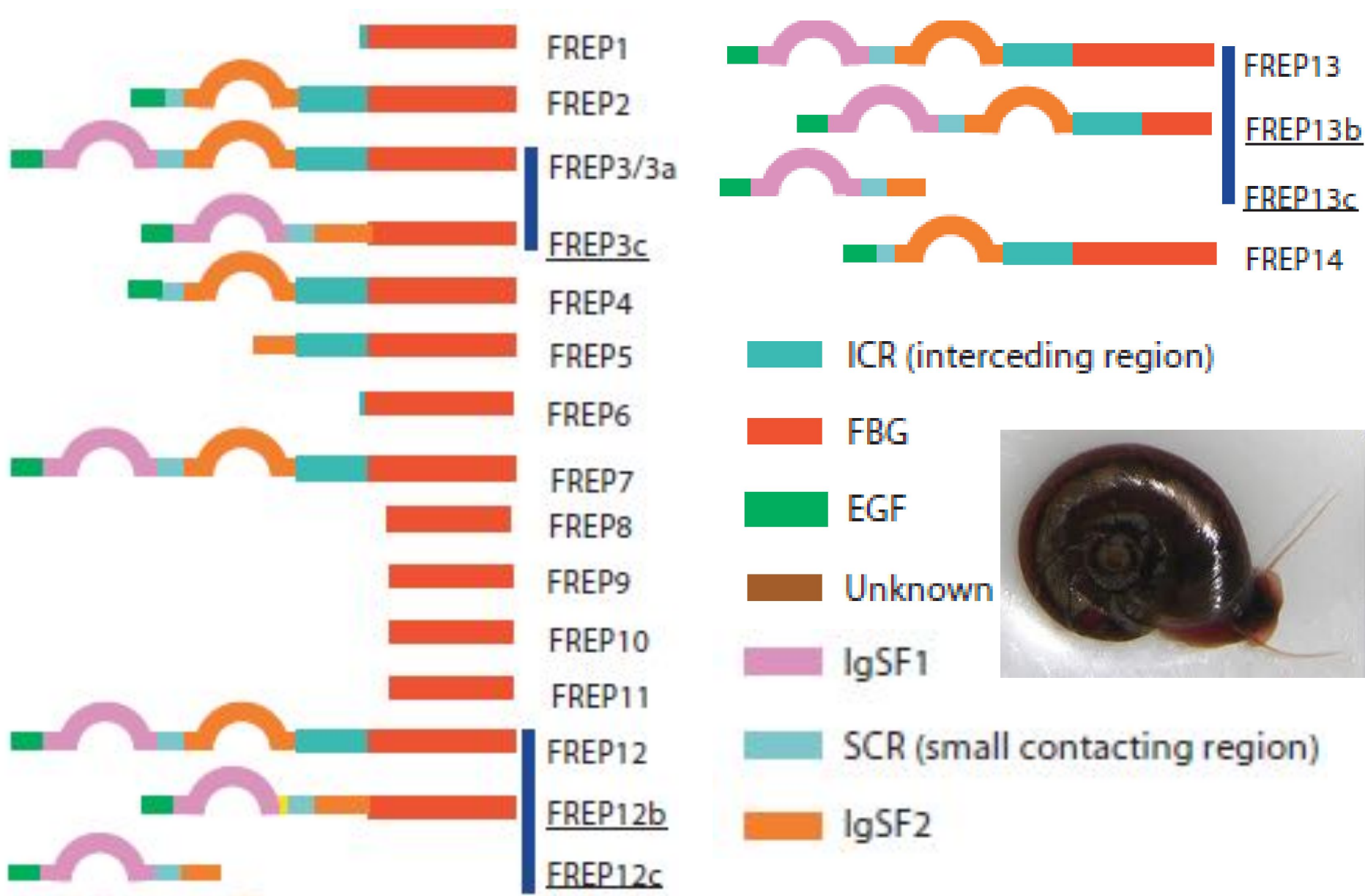
Lumbricin like, **cationic**, anti Gram + and Gram -



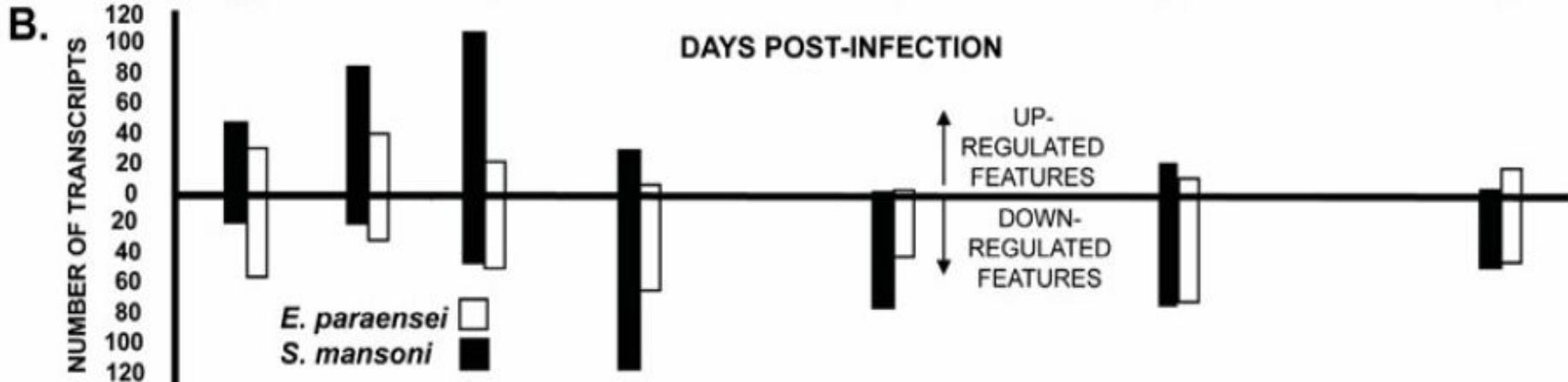
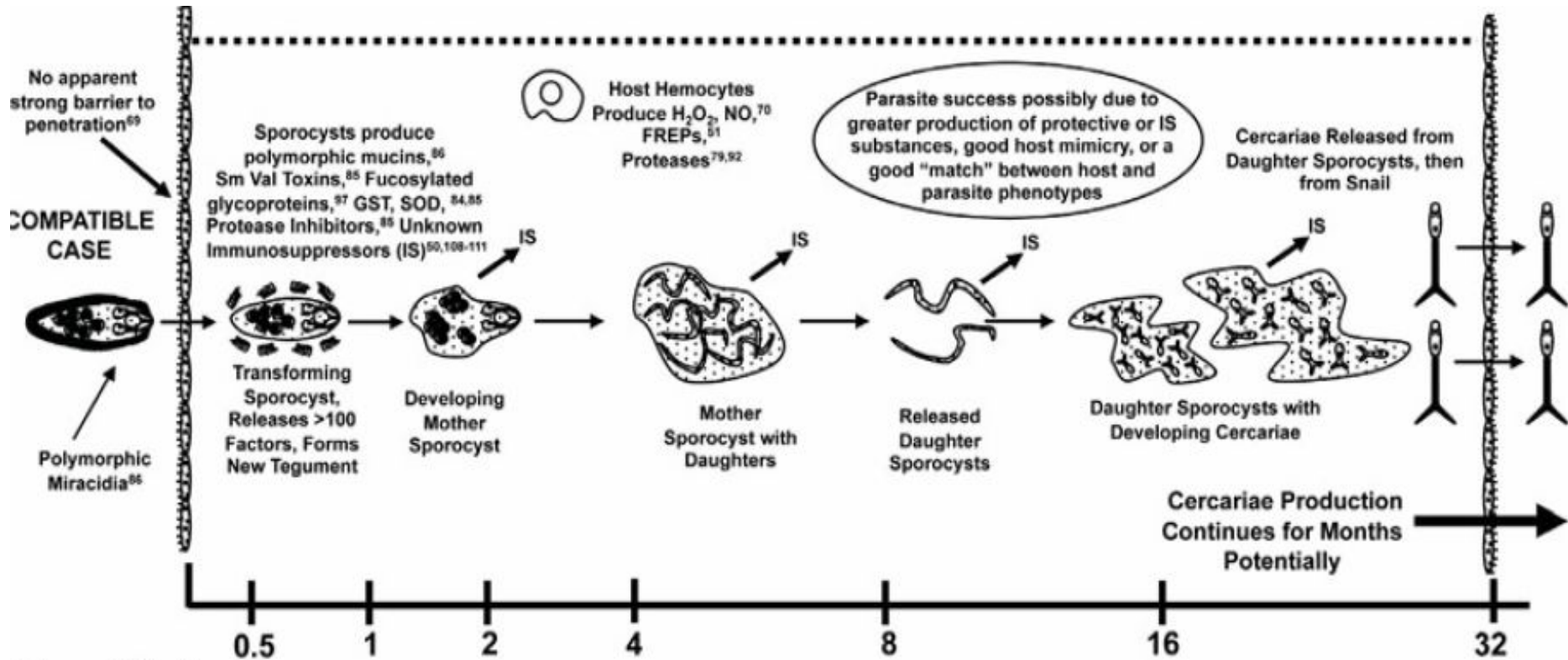
Peptide B, **anionic**, anti Gram +



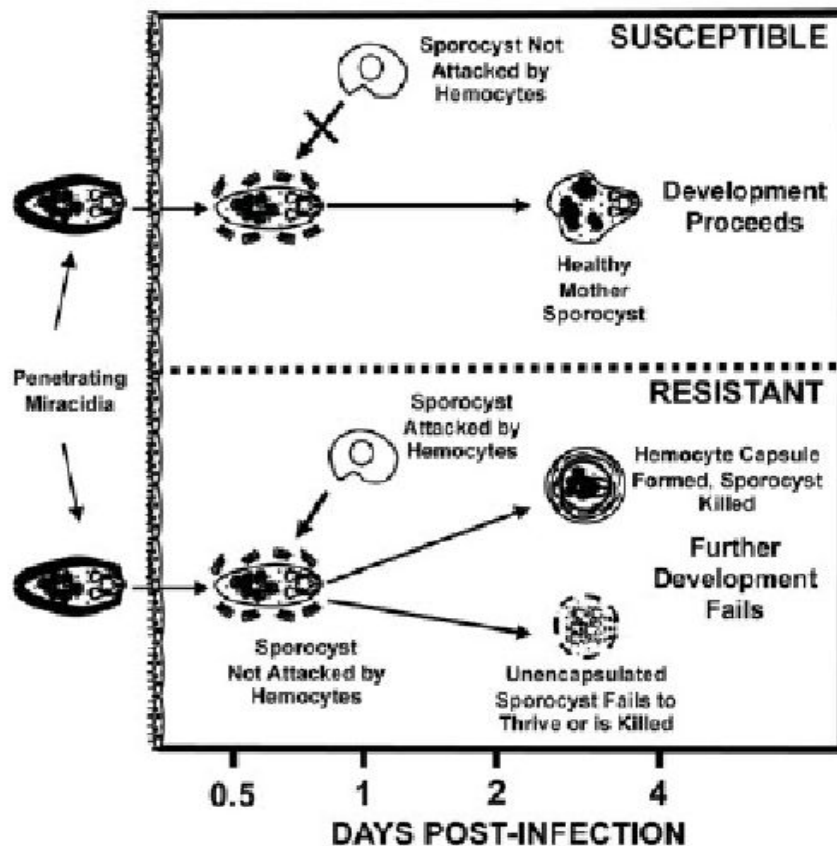
# Строение FREP белков улитки *Biomphalaria glabrata*



# Улитки против трематод



# Различия между чувствительными и устойчивыми улитками



## CONSTITUTIVE DIFFERENCES BETWEEN SUSCEPTIBLE AND RESISTANT SNAILS

Resistant Snails have more hemocytes than susceptible snails

Bg-Sm: 179

Resistant hemocytes have different behavior or properties

Bg-Sm: 177

Bt-Sm: 178

Resistant Snails produce more parasite-toxic reactive oxygen species (ROS)

Bg-Sm: 70,101,179-182

Resistant Snails have novel recognition/killing capacity in their plasma

Bt-Sm: 183,184

Bg-Sm: 44

Innate differences in the levels of potentially immune relevant factors

Bg-Sm: 185

Bg-Ec: 73,79,84,186,187

Bt-Sm: 188

Resistant snails have greater ability to resist parasite offensive strategies, including signaling pathway targets

Bg-Sm: 43,110

Bg-Ec: 119

Resistant and susceptible snails may differ in the ease with which they are mimicked by parasites

Bg-Sm: 97

## RESPONSE CAPACITIES FOLLOWING EXPOSURE TO INFECTION

Resistant Snails are more stimulated to make hemocytes following exposure

Bg-Sm: 189

Resistant snails respond more prominently or differently to infection than susceptible snails

Bg-Sm: 92,100,102,103,105,100-101

Bg-Ec: 73,74,77,84,187

Bt-Sm: 188

# Гипотеза черной королевы

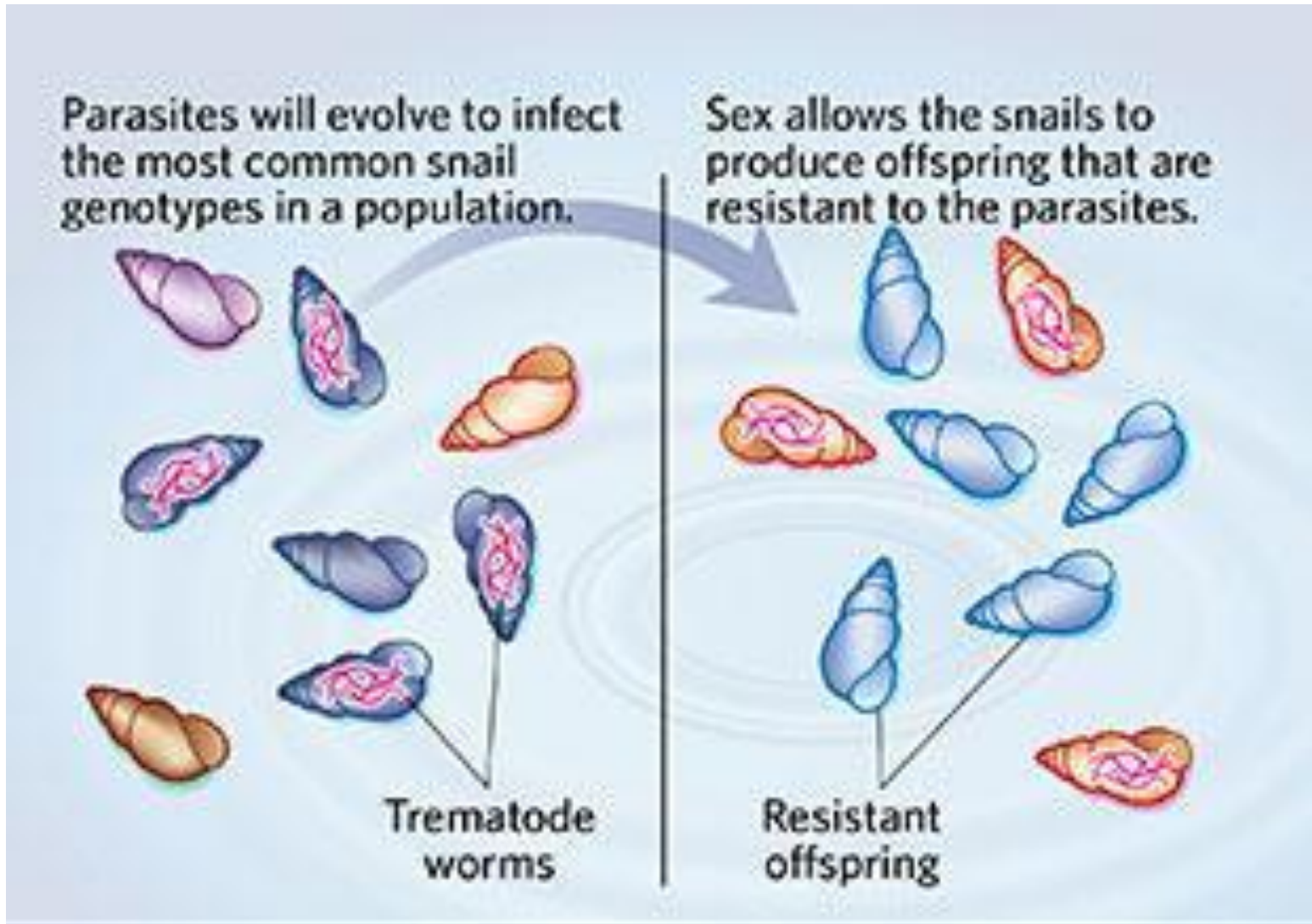
## RED QUEEN HYPOTHESIS

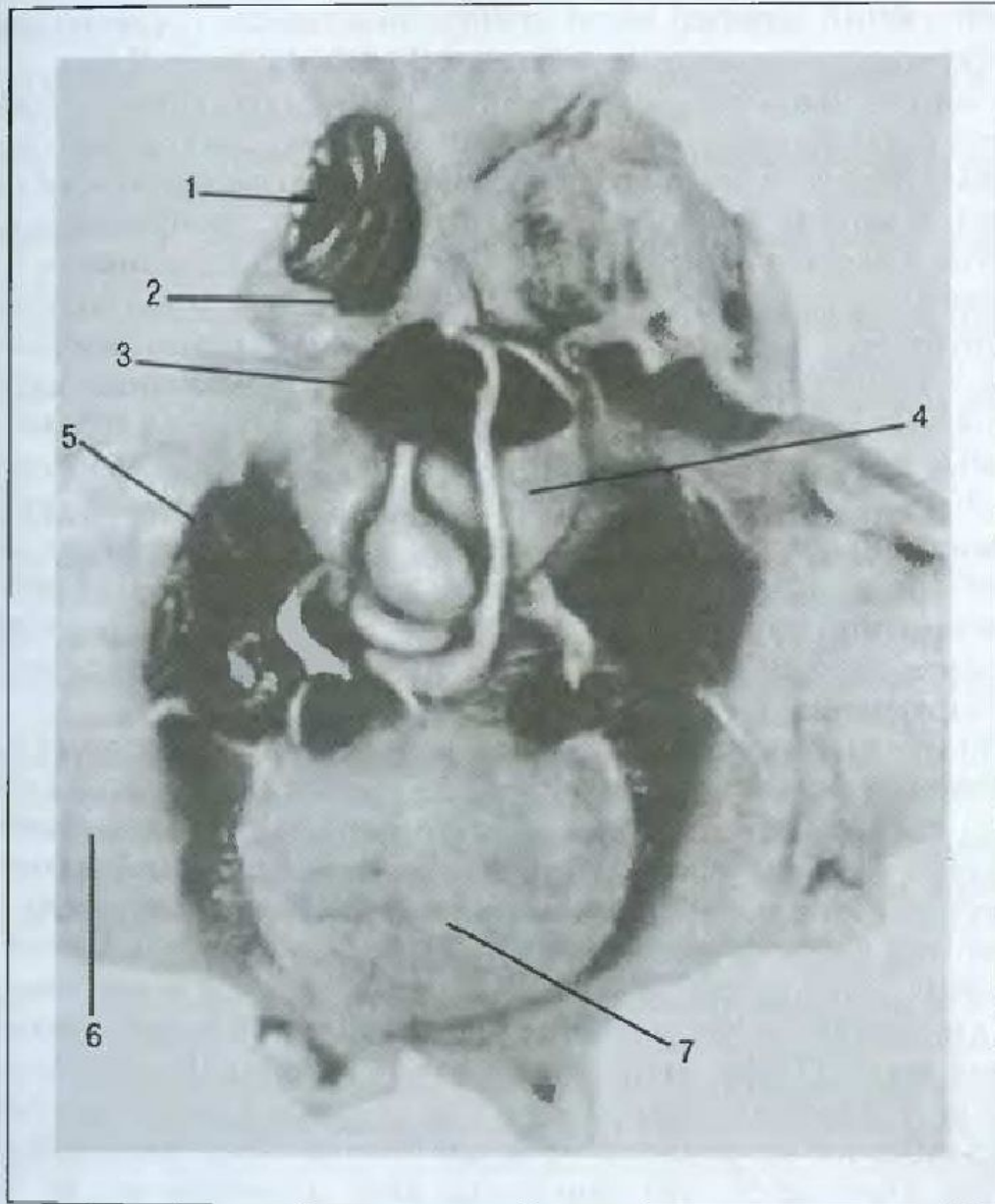


IN REFERENCE TO AN EVOLUTIONARY SYSTEM,  
CONTINUING ADAPTATION IS NEEDED IN ORDER  
FOR A SPECIES TO MAINTAIN ITS RELATIVE FITNESS  
AMONGST THE SYSTEMS IT IS CO-EVOLVING WITH

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# Половое размножение помогает устойчивости к паразитам на популяционном уровне





*Euprymna scolopes*

Рис. 7.7. Диссекция осьминога *Eledone ceratosa* через 2 дня после внутривенной инъекции большой дозы туши

Лимфоидный орган — белое тело (2) — расположен непосредственно за глазом (1);  
 3 — задняя слюнная железа; 4 — печень; 5 — жабры; 6 — мантия; 7 — гонады



# Строение светового органа эупримны

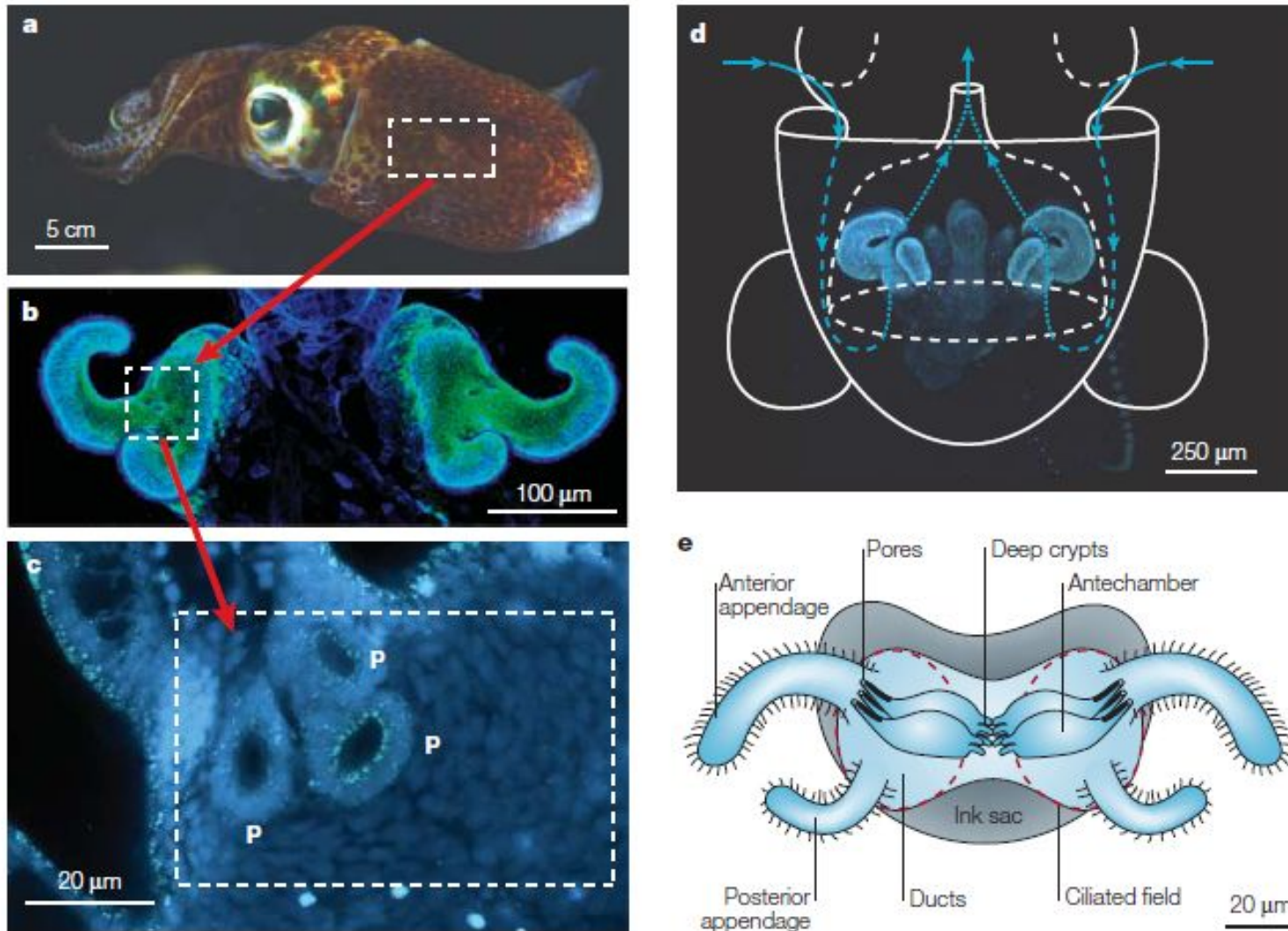
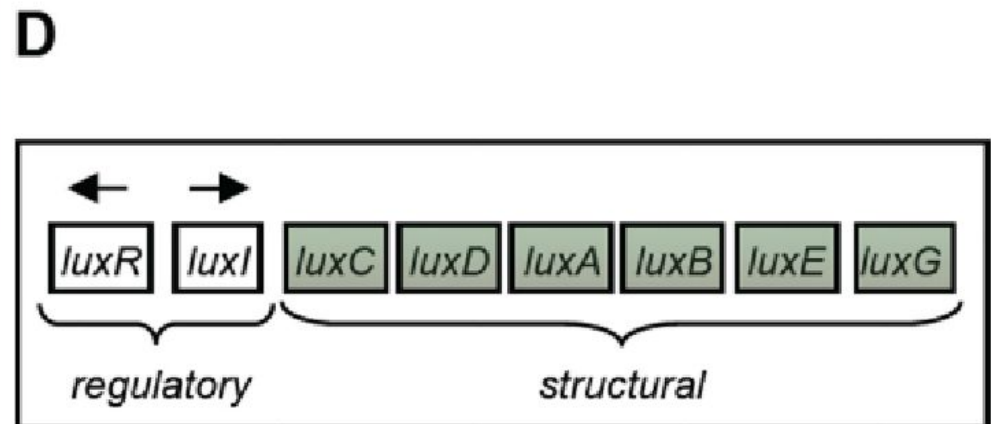
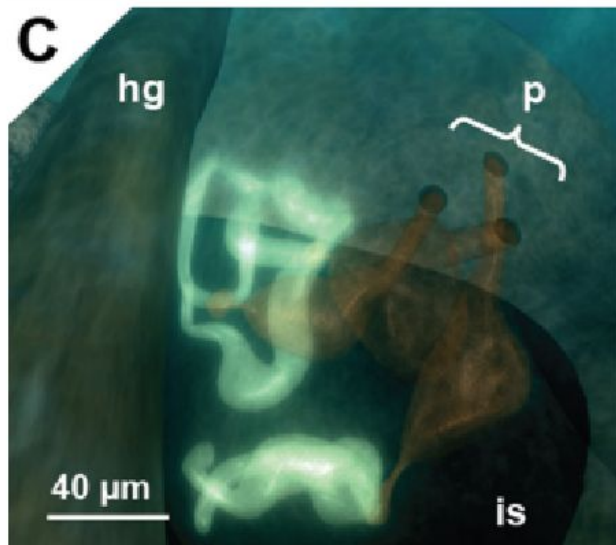
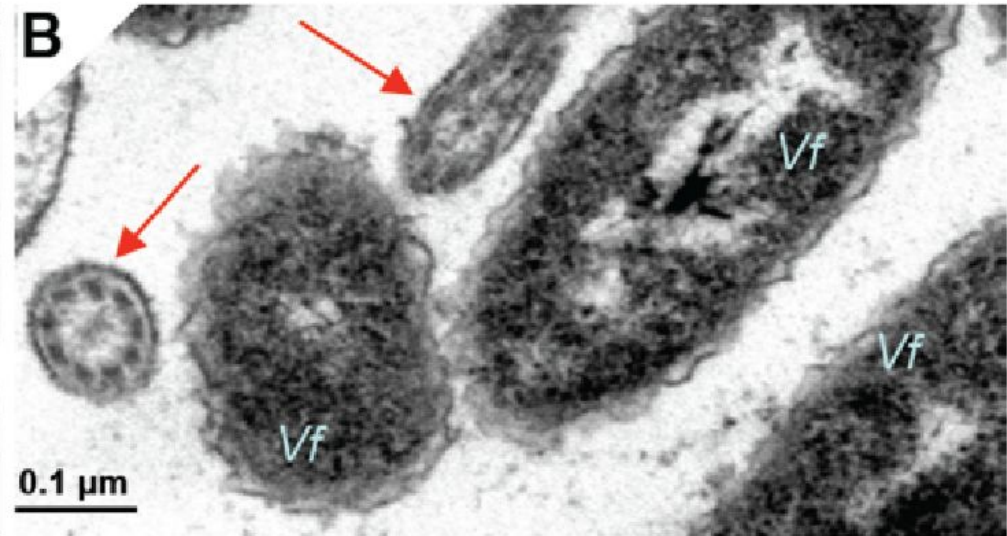
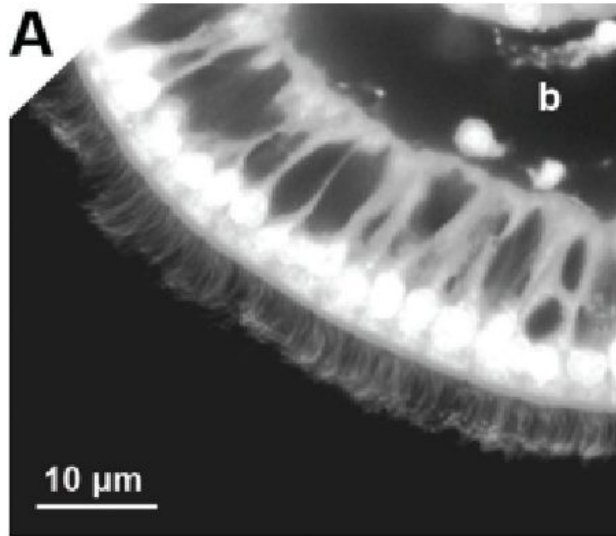


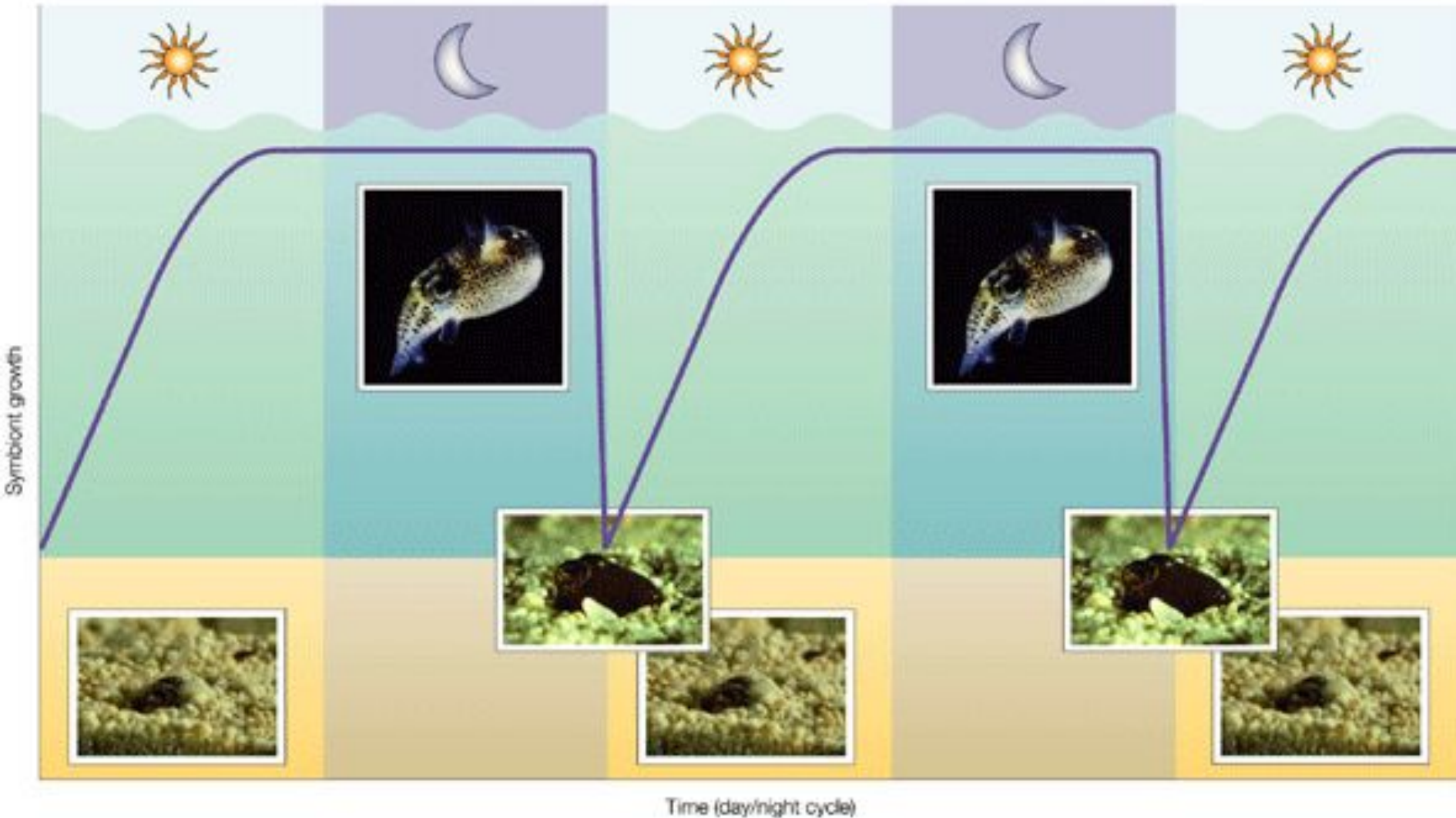
Figure 2 | **The juvenile light-organ system.** **a** | A host squid swimming in the water column. **b** | A confocal micrograph showing the complex ciliated epithelium on the surface of the juvenile light organ. **c** | A confocal micrograph showing the pores (P) on the surface of the organ through which the symbionts enter host tissues. **d** | The pattern of water flow through the mantle cavity. **e** | The internal components of the squid light organ at hatching. Part **d** is reproduced with permission from REE. 18 © (2000) National Academy of Sciences.

# *Vibrio fischeri* прикрепляются к ресничному эпителию светового органа каракатицы

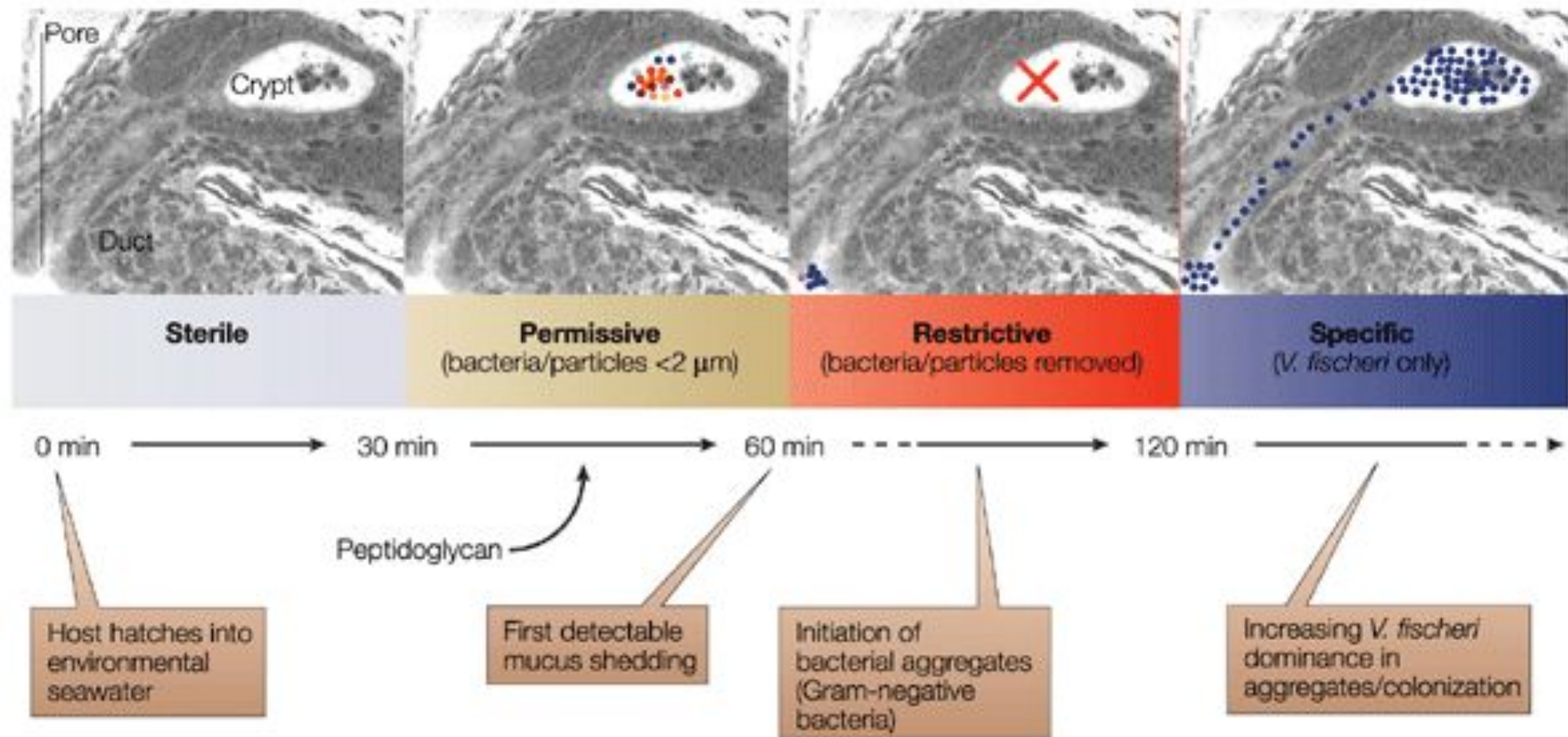


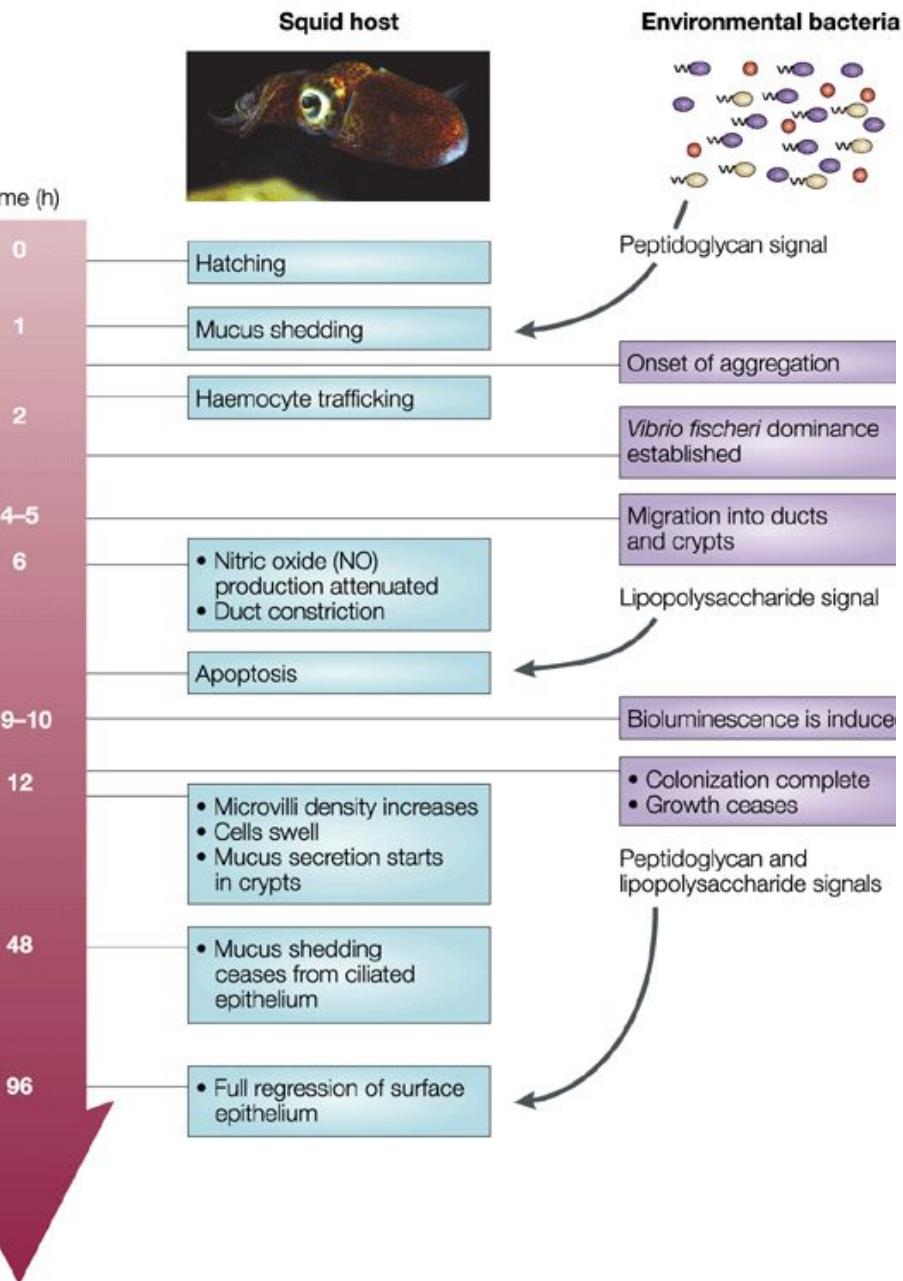
Интенсивность свечения  
зависит от чувства кворума

# Каракатица регулирует количество симбионтов в световом органе



# Заселение светового органа каракатицы после рождения





## Модель колонизации

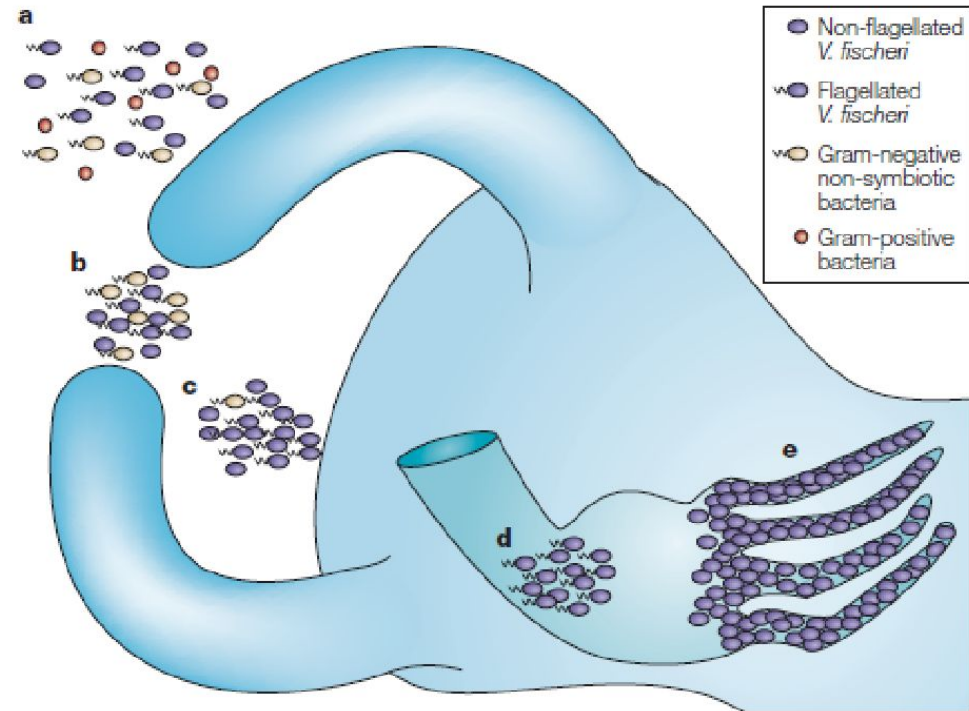


Figure 8 | **The winnowing.** This model depicts the progression of light-organ colonization as a series of steps, each more specific for symbiosis-competent *Vibrio fischeri*. **a** | In response to Gram-positive and Gram-negative bacteria (alive or dead) the bacterial peptidoglycan signal causes the cells of the ciliated surface epithelium to secrete mucus. **b** | Only viable Gram-negative bacteria form dense aggregations. **c** | Motile or non-motile *V. fischeri* out-compete other Gram-negative bacteria for space and become dominant in the aggregations. **d** | Viable and motile *V. fischeri* are the only bacteria that are able to migrate through the pores and into the ducts to colonize host tissue. **e** | Following successful colonization, symbiotic bacterial cells become non-motile and induce host-epithelial cell swelling. Only bioluminescent *V. fischeri* will sustain long-term colonization of the crypt epithelium.

# Деградация ресничного эпителия светового органа идет в ответ на МАРП *Vibrio fischeri*

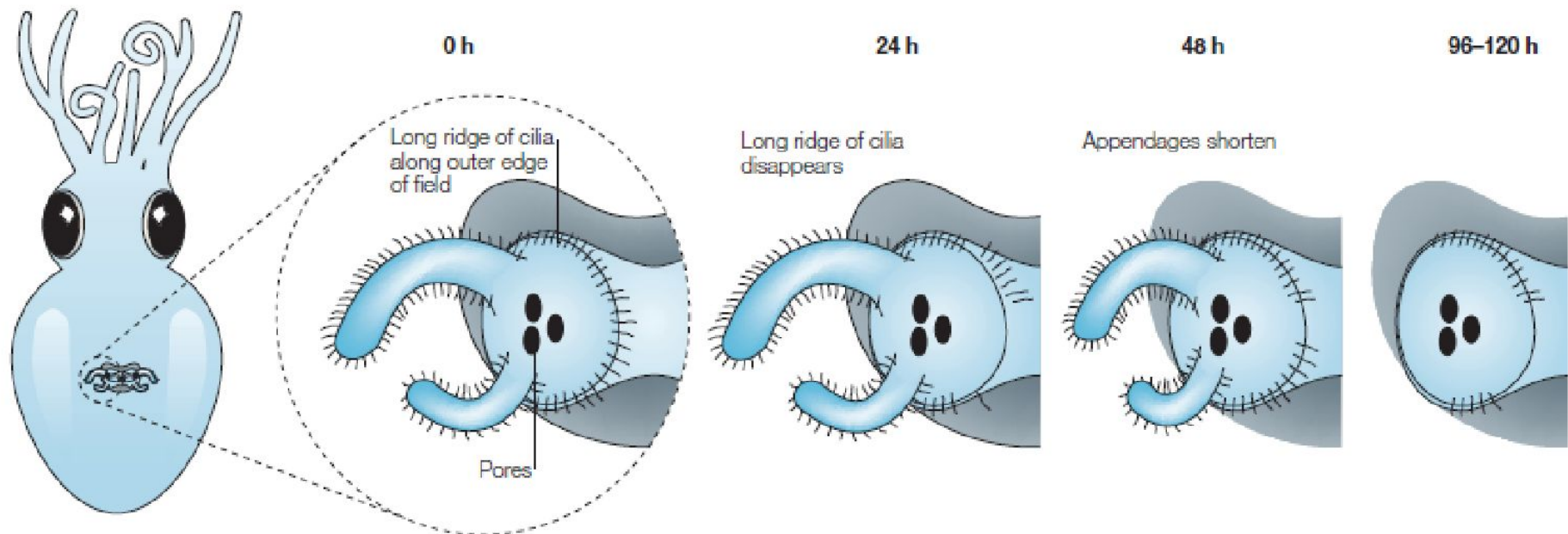
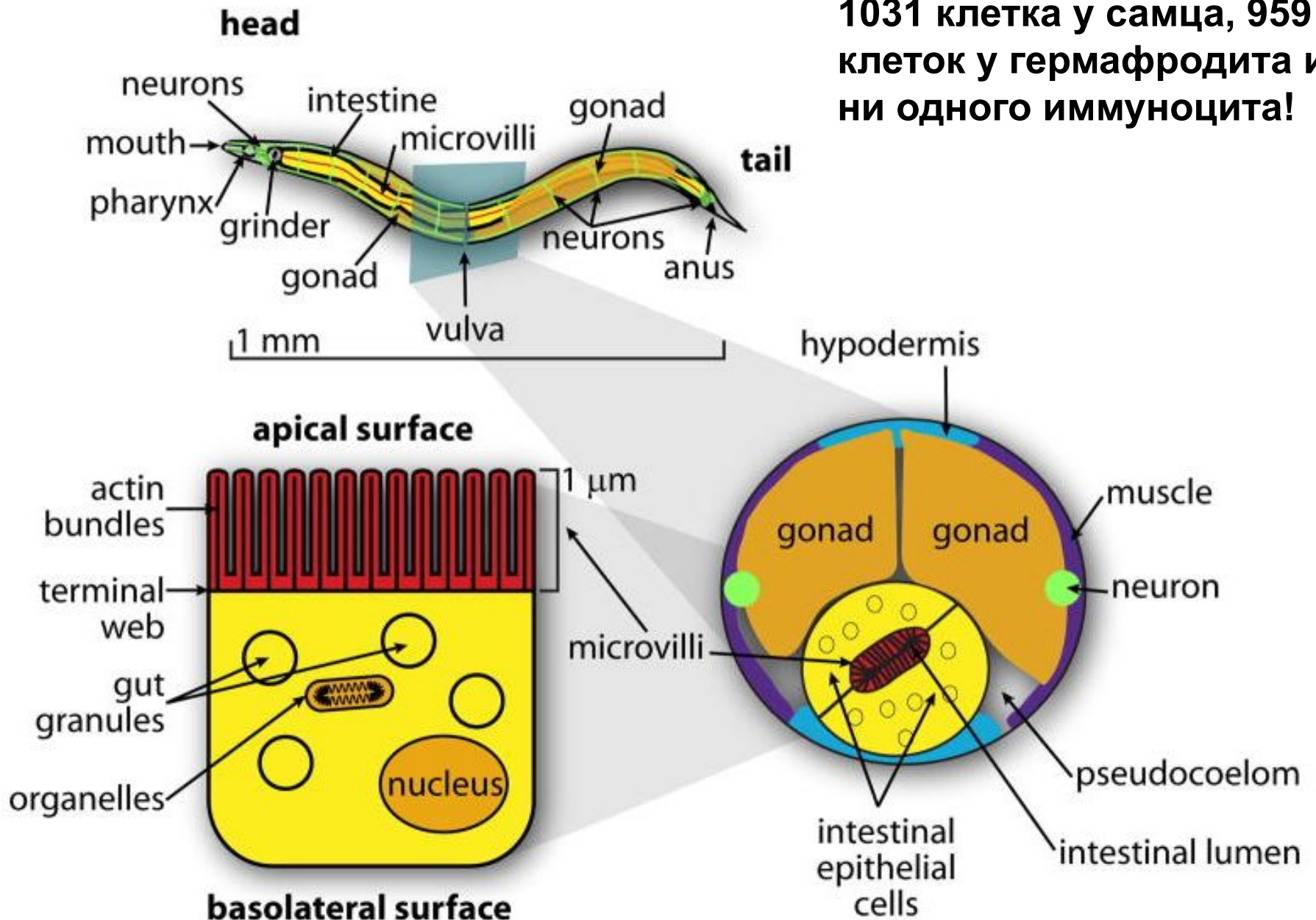


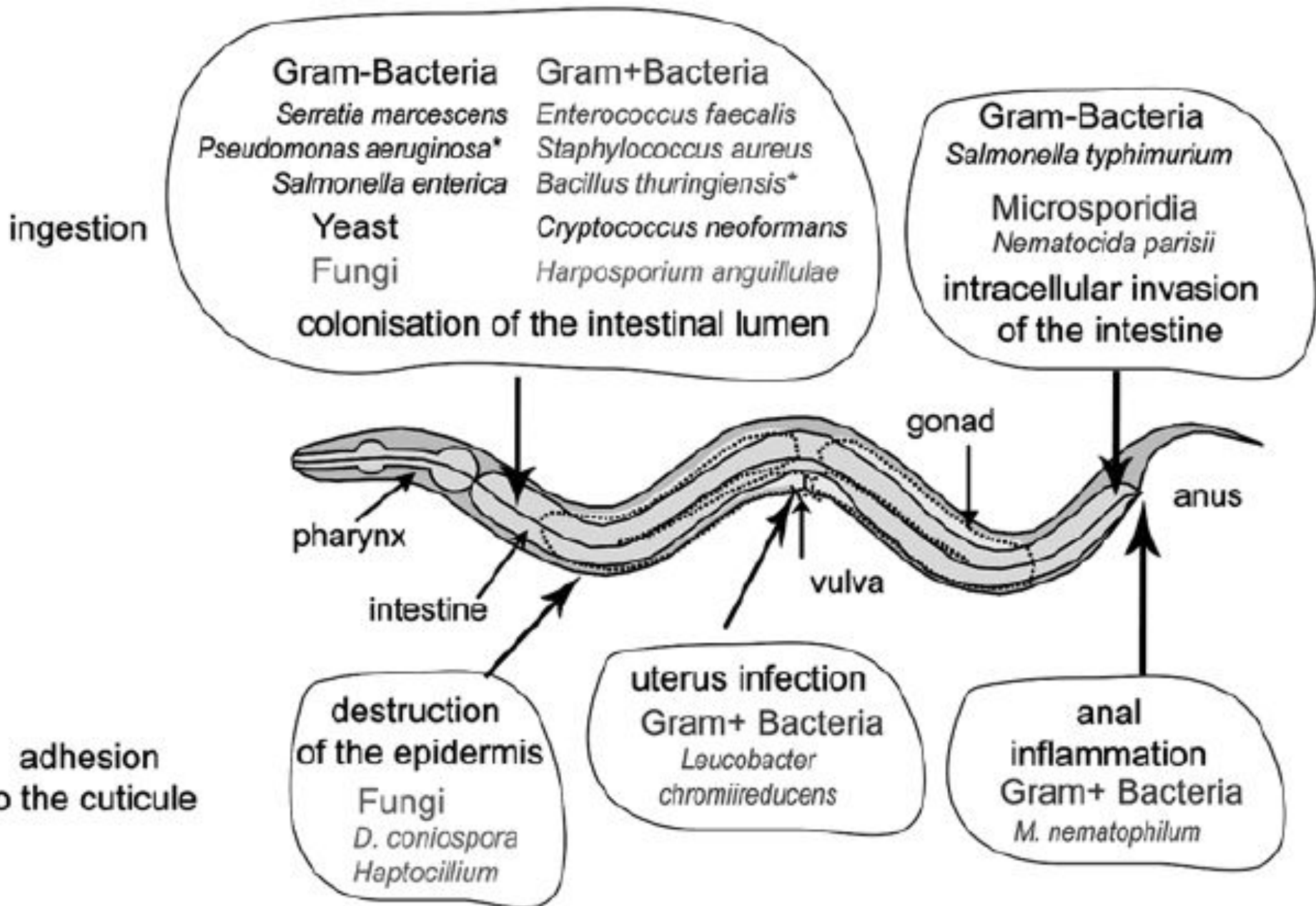
Figure 5 | The gradual, symbiont-induced regression of the ciliated epithelium of the juvenile light organ. Schematic depicting the loss of host ciliated epithelial fields after successful colonization by *Vibrio fischeri*.

# Общее строение нематоды

1031 клетка у самца, 959 клеток у гермафродита и ни одного иммунита!

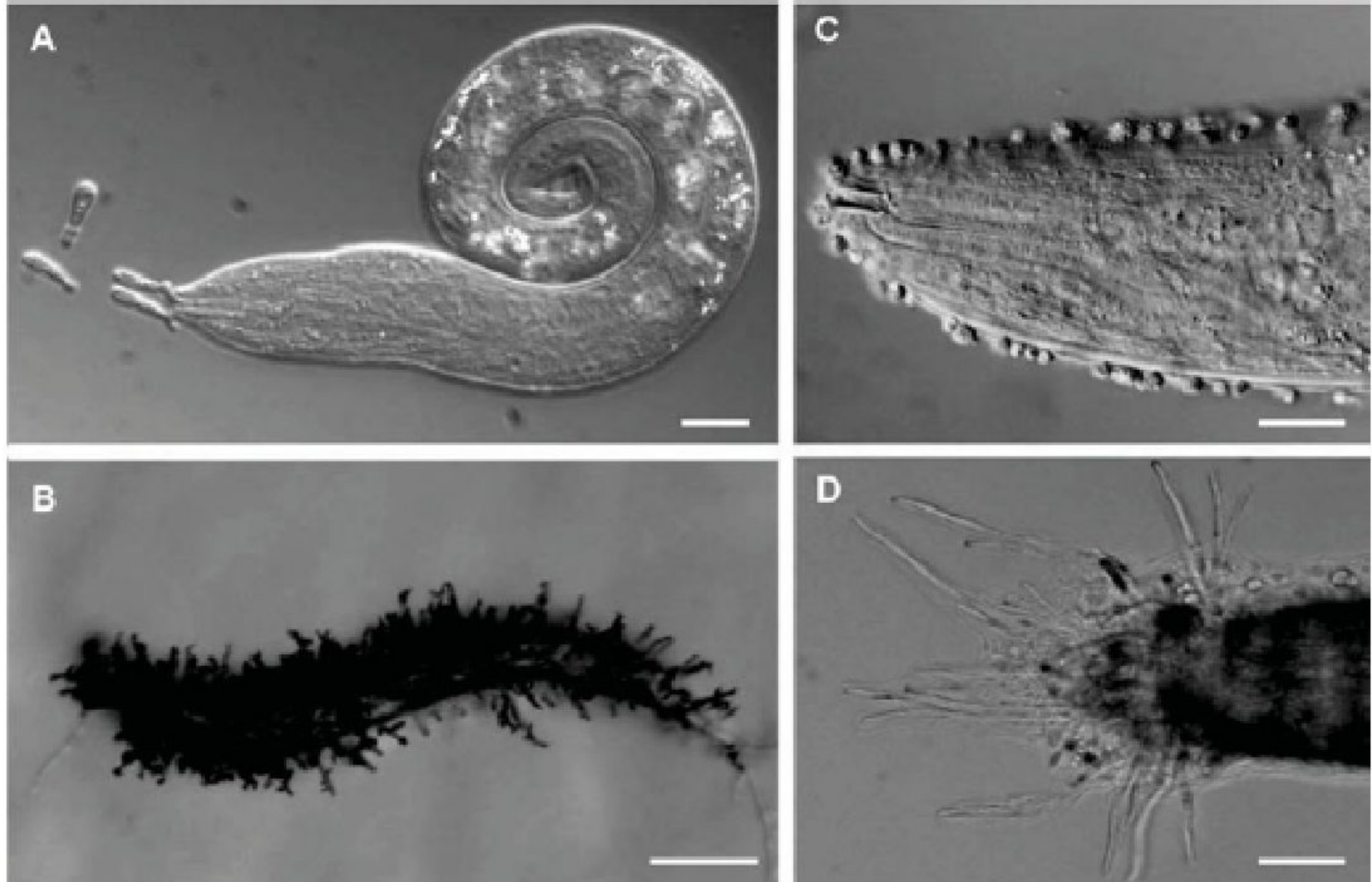


# Патогены нематод и пути их проникновения



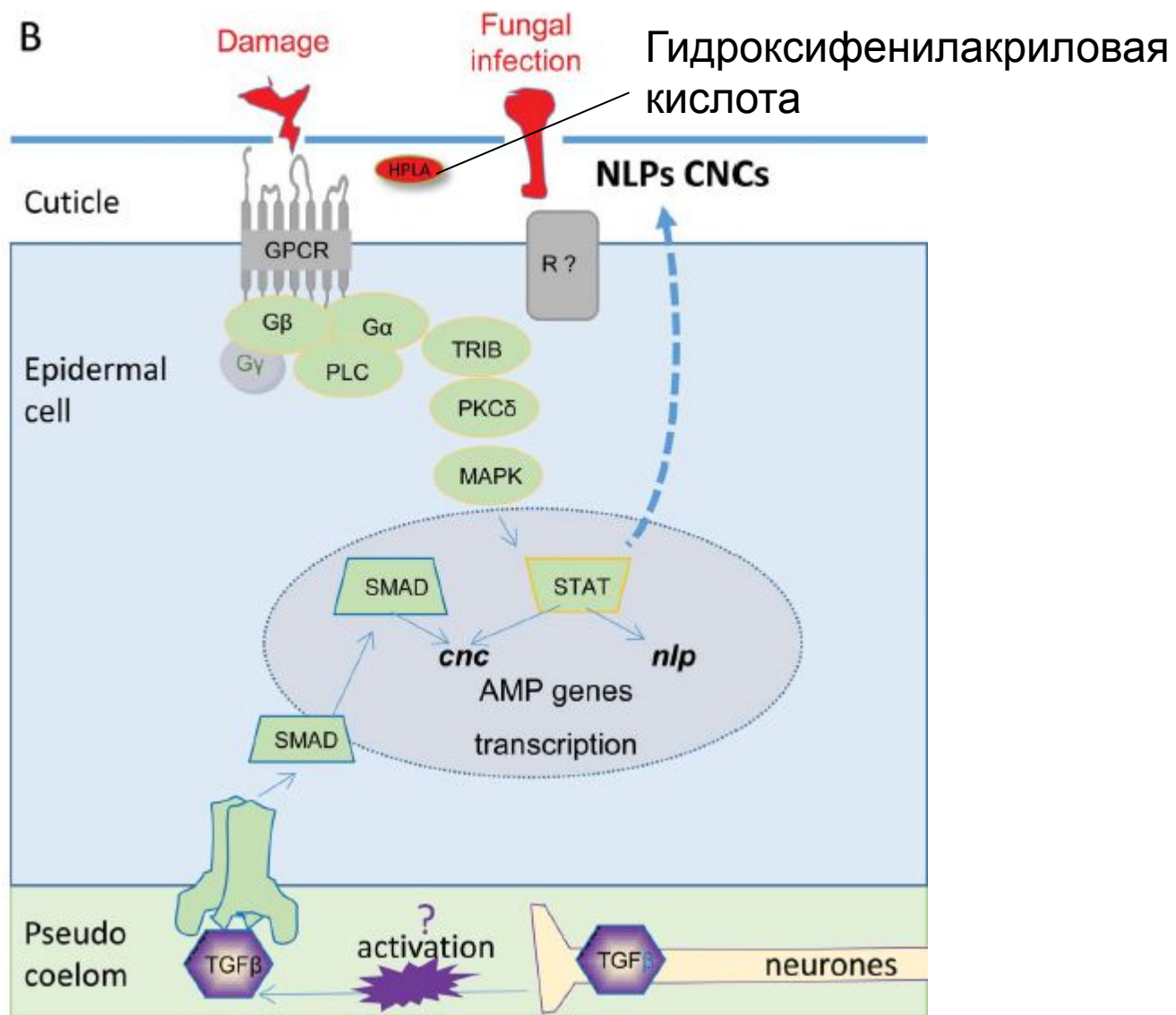


# Грибковые инфекции нематод

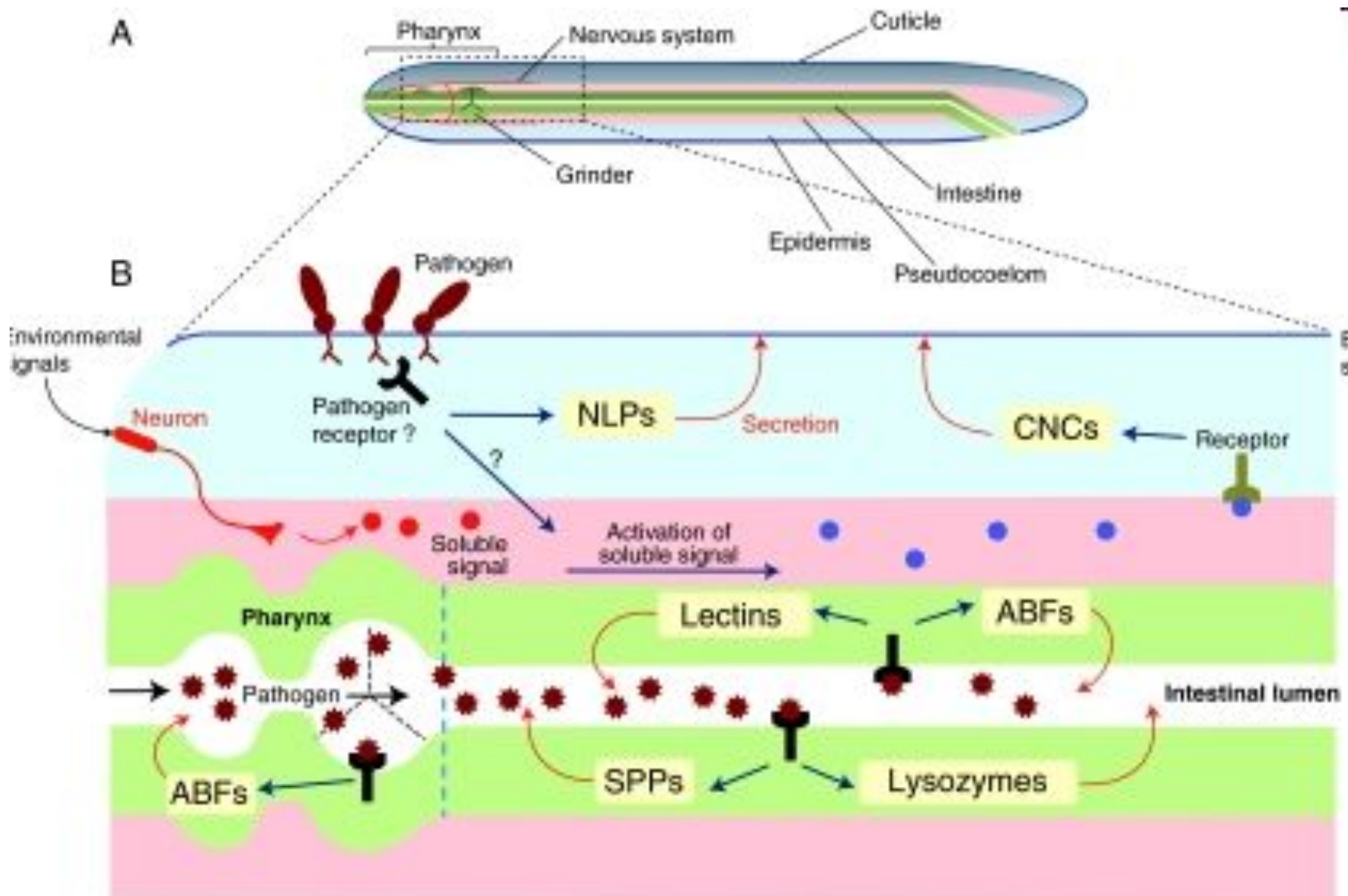


**Figure 2.** Fungal infection of *C. elegans*. (A and B) *D. coniospora*, (C and D) *Haptocillium*. (A and C) adhesion of the spores to the cuticle after few hours, (B and D) after 2 days fungal hyphae grow out of the worm. Scale bars are 10  $\mu\text{m}$  (A), 100  $\mu\text{m}$  (B) and 50  $\mu\text{m}$  (C and D).

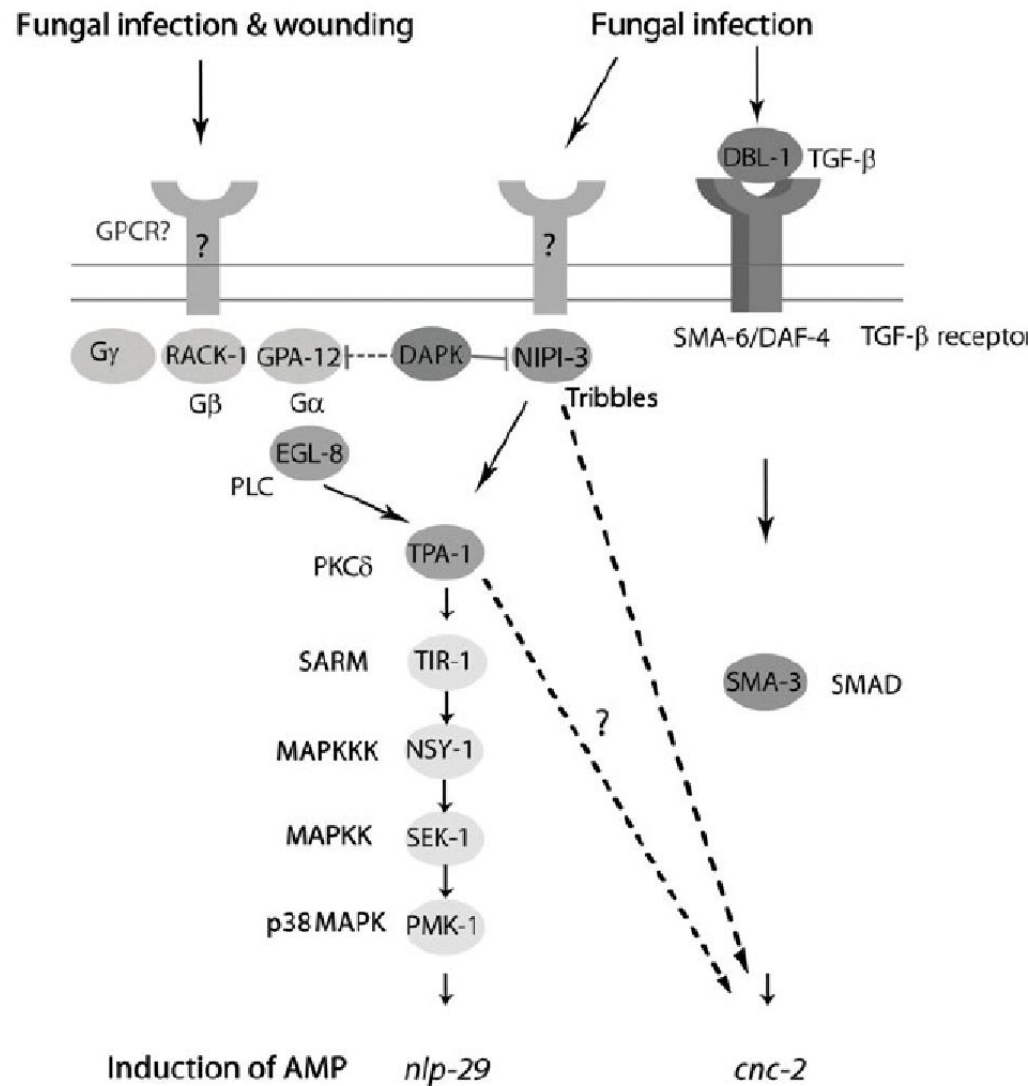
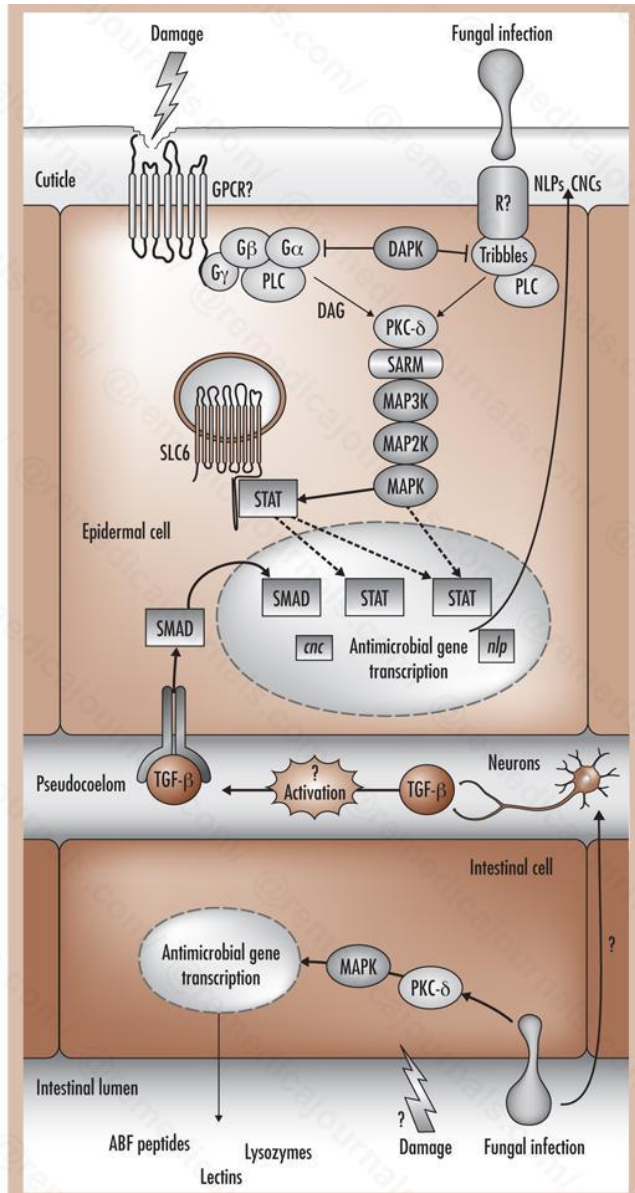
# MAP-киназный каскад как сигнал о повреждении покровов



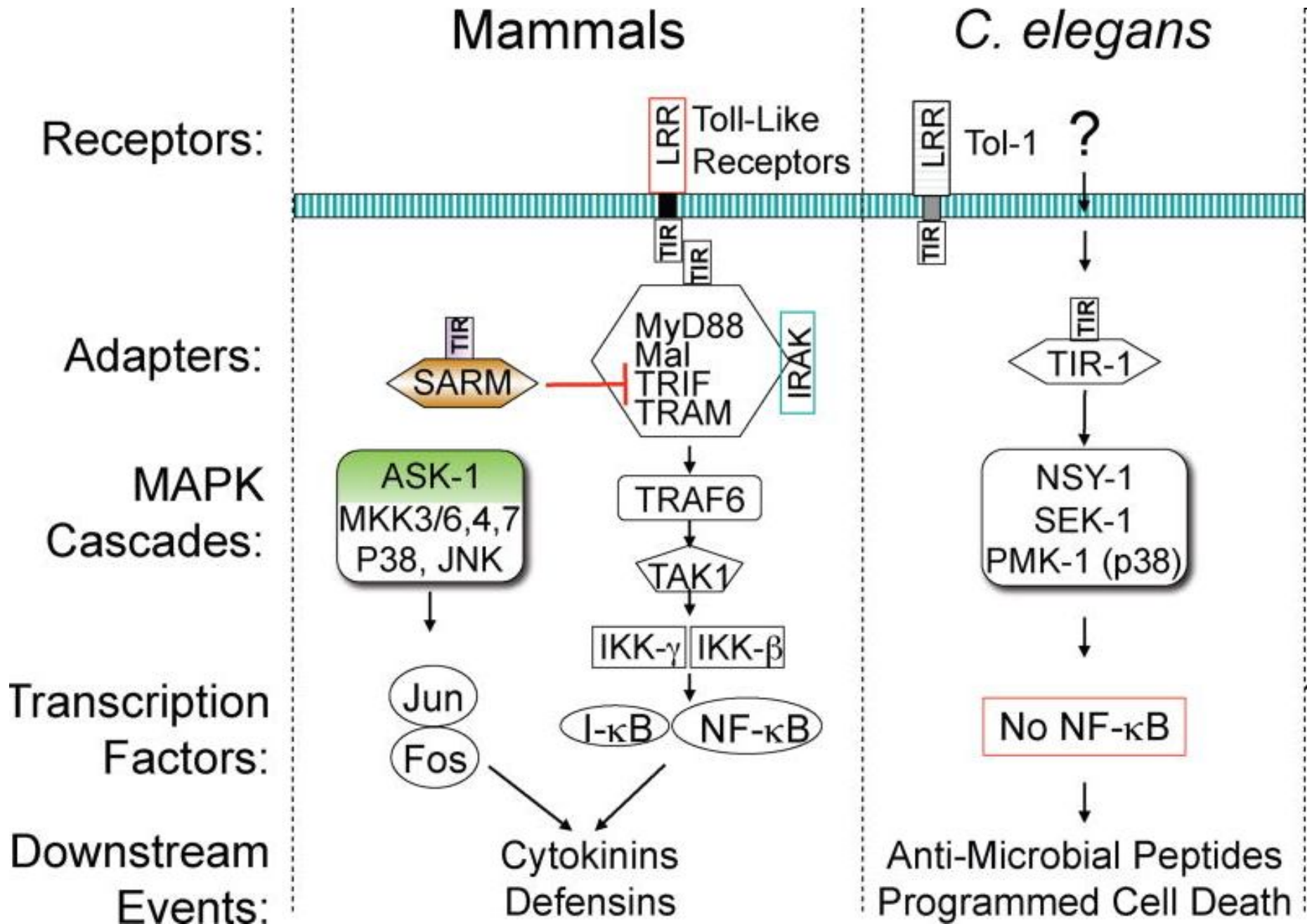
# Иммунная система нематод



# Сигнальные пути, вовлеченные в иммунный ответ нематоды, еще не известны полностью



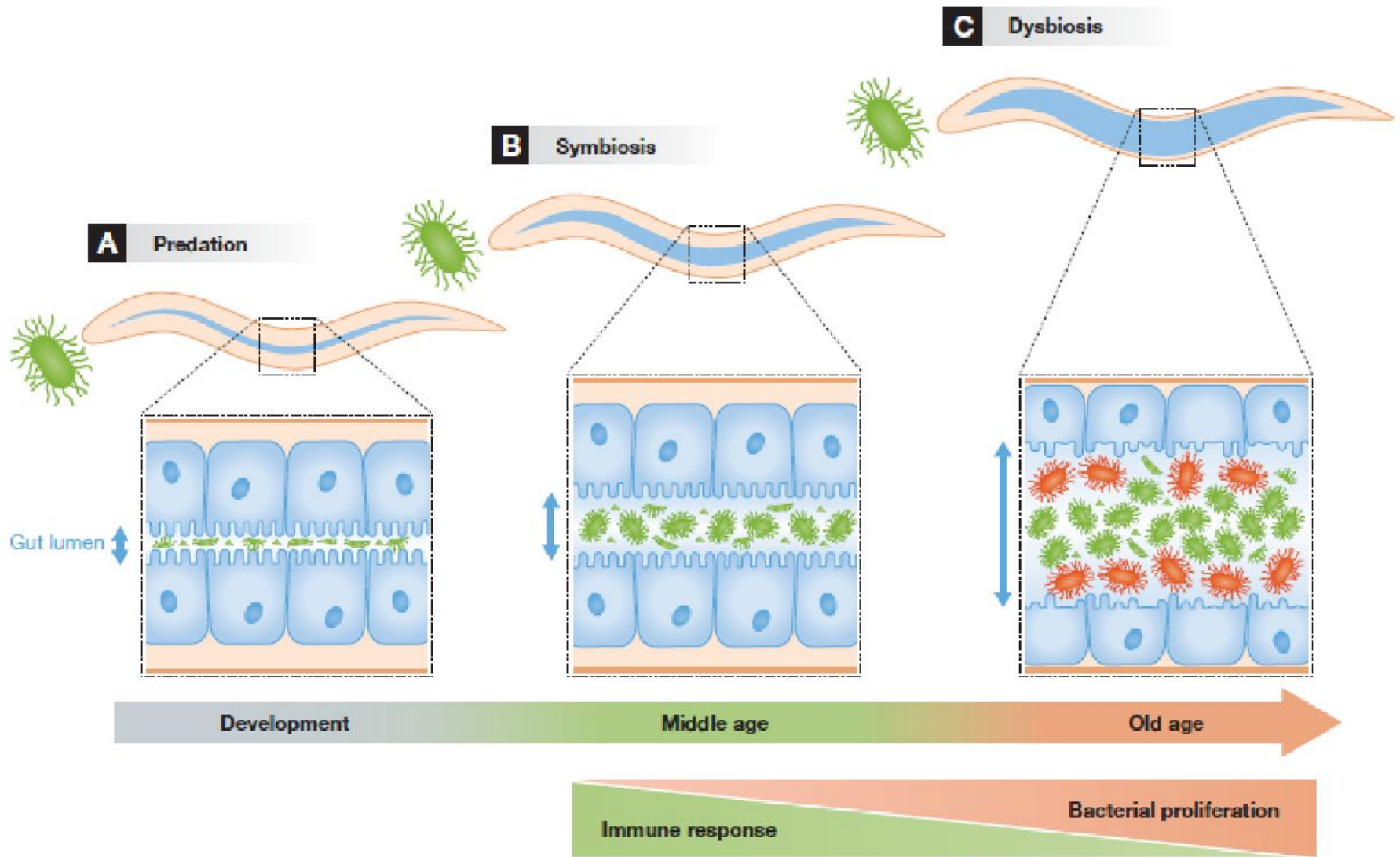
# Различие сигнальных путей



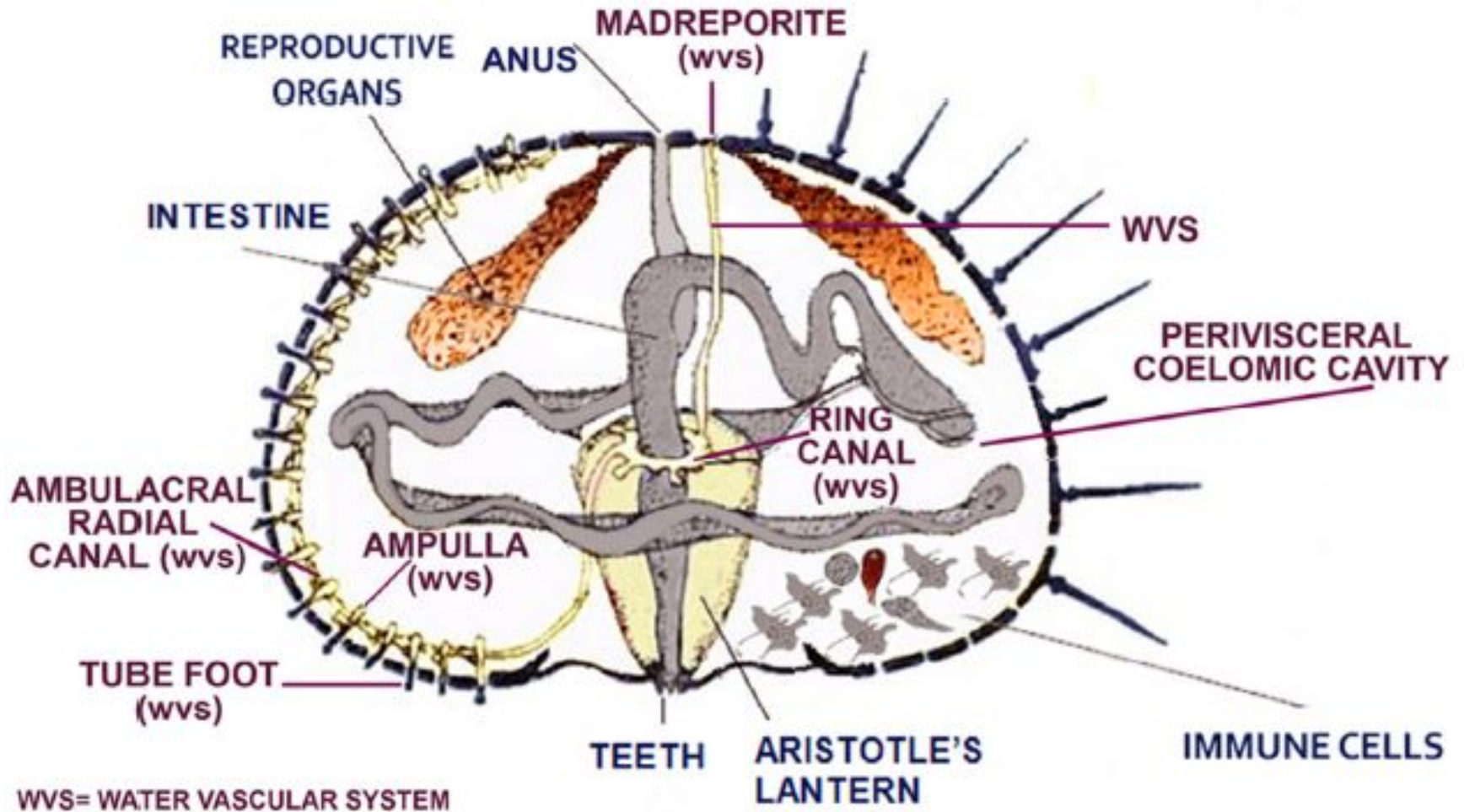
**Table 1.** Summary of the major signalling pathways in the *C. elegans* Immune System (updated from ref. 86)

Pathway	Tissue	Components	Homologues	References
p38 MAPK	Epidermis	GPA-12, RACK-1	G protein subunits	82
		EGL-8, PLC-3	Phospholipase C	82
		NIPI-3	Tribbles kinase	35
	Epidermis and intestine	TPA-1	Protein kinase C	82,87
TIR-1		SARM	24,34,88	
NSY-1, SEK-1, PMK-1		MAP kinases	12,35	
FSHR-1	Intestine	FSHR-1	G protein coupled receptor	26
ZIP-2	Intestine	ZIP-2	b-zip transcription factor	65
Insulin signalling	Nervous system	INS-7	Insulin-like peptide	76
	Intestine	DAF-2	Insulin receptor	42
		AGE-1	PI3 kinase	42
		AKT-1, AKT-2	Akt kinase	43
		DAF-16	FOXO transcription factor	42
TGF- $\beta$	Nervous system epidermis	DBL-1	TGF- $\beta$	54,55
		SMA-6	TGF- $\beta$ receptor	55
		SMA-3	SMAD protein	55
Wnt/Hox	Intestine/	BAR-1	$\beta$ -catenin	61
	Hindgut	EGL-5	Hox transcription factor	61,64
ERK MAPK	Hindgut	LIN-45, MEK-2, MPK-1	ERK MAP kinase	39
		EGL-8	Phospholipase C	89
		SUR-2	Mediator component	39
UPR <sup>1</sup>	Intestine	XBP-1	X box protein	50,52
		HSP-4	Heat shock protein	
Autophagy	Pharynx	CED-1, C03F11.3	Scavenger receptor	51
	Intestine	BEC-1, LGG-1	ATG proteins	16

# Микробиота нематоды и старение



# Общая схема строения морского ежа

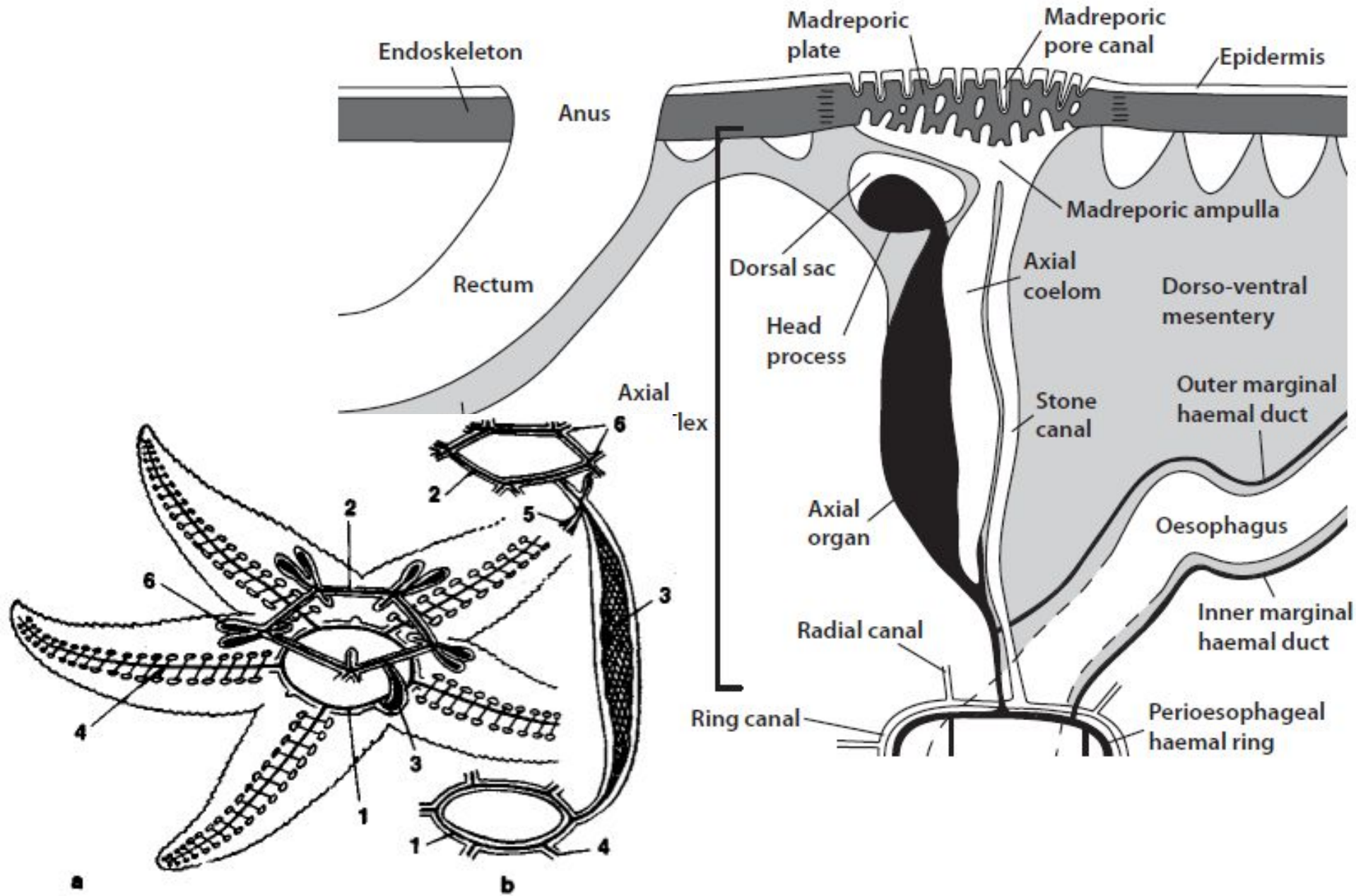




# Характеристика генов иммунной системы иглокожих и хордовых

	Amphioxus <sup>a</sup>	Sea squirt <sup>b</sup>	Sea urchin <sup>c</sup>	Human <sup>d</sup>
Complement				
C3/C4/C5 <sup>e</sup>	3	2	4	4
Bf/C2 <sup>f</sup>	2	3	3	2
MASP <sup>g</sup> /Clr,s <sup>h</sup>	5/44 <sup>i</sup>	4	0/2 <sup>l</sup>	4
TCC <sup>l</sup>	9	11	0	6
Clq <sup>k</sup> -like	39	2	4	23
LRR-containing				
TLR <sup>l</sup>	72	2	222	10
NLR <sup>m</sup>	118	0/28 <sup>n</sup>	203	25
LRR-Ig <sup>o</sup>	125	ND <sup>p</sup>	22	30
Other mediators				
SRCR <sup>q</sup>	270	5	218	16
PGRP <sup>r</sup>	18	0	5	6
GNBP <sup>s</sup>	5	3	3	0
CTL <sup>t</sup>	1200/717 <sup>u</sup>	ND <sup>p</sup>	104	81
VCBP <sup>v</sup>	5/10 <sup>w</sup>	4	0	0
Cytokines				
TNF <sup>x</sup>	21	4	4	20
TNFR <sup>y</sup>	31	3	9	26
IL-17 <sup>z</sup>	9	2	30	9

# Аксиальный орган иглокожих

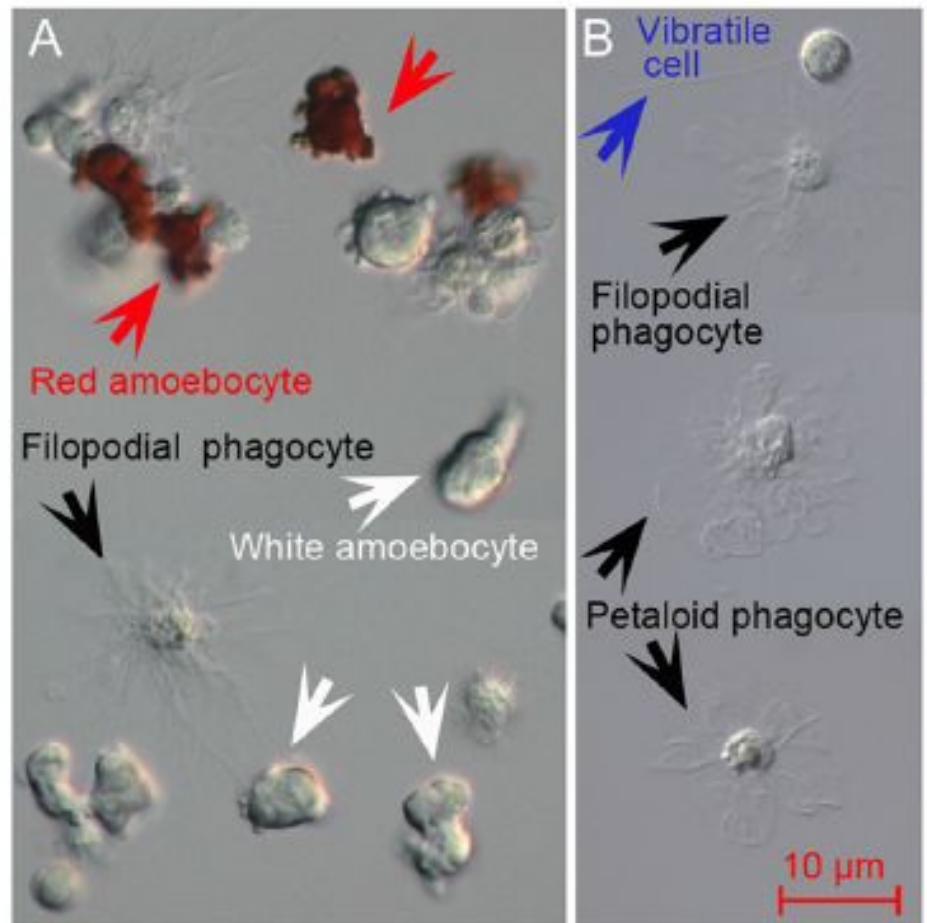


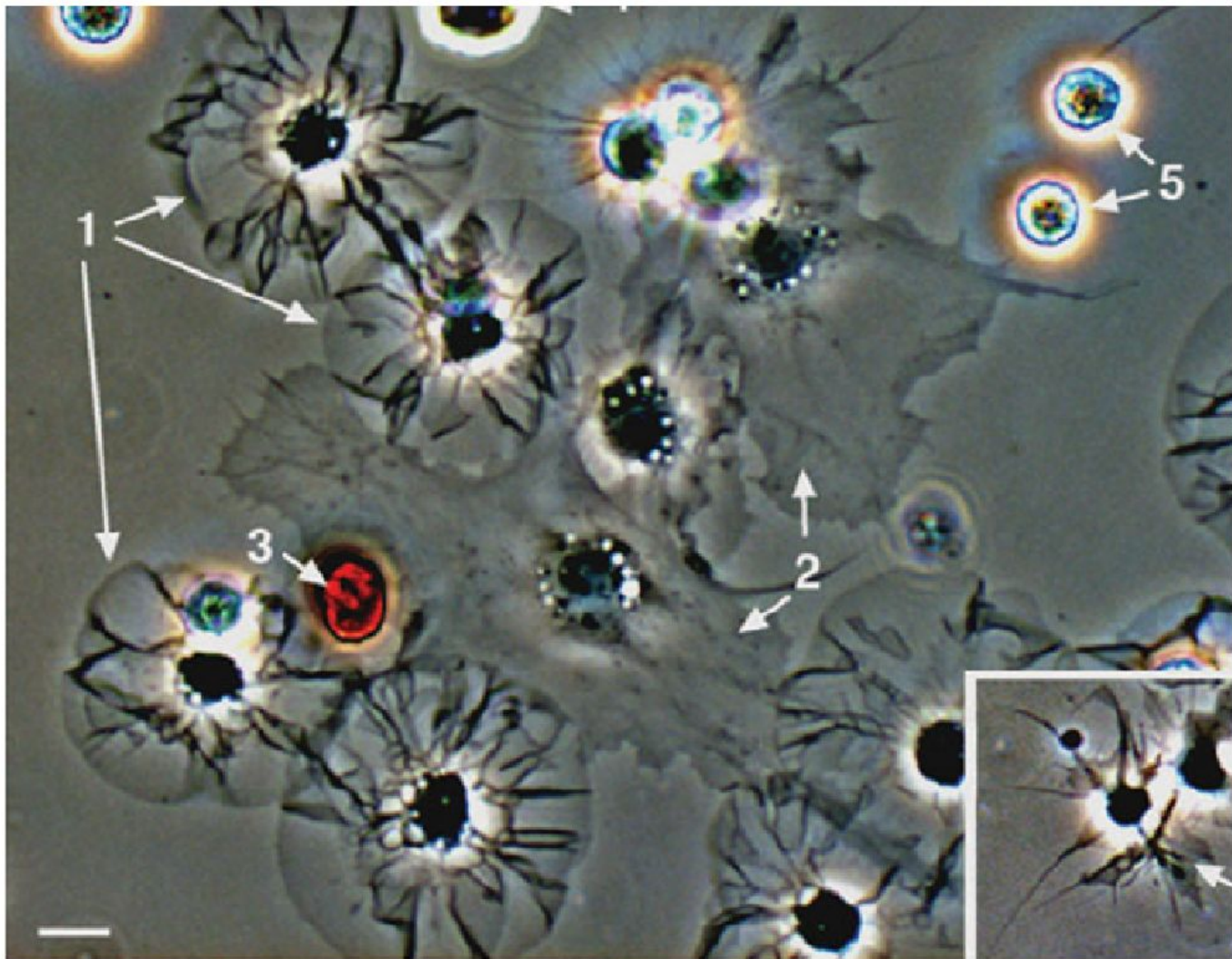
# «Формула» целомоцитов морского ежа

Table 1. Coelomocytes in sea urchins

Cell Type	% in Coelomic Fluid	Function
Phagocyte Types	Total phagocytes	Encapsulation, Opsonization, Graft rejection, Chemotaxis, Phagocytosis, Antibacterial activity, Cellular clotting
Type 1—Discoidal cells	<i>Sp</i> * 40-80%	
Type 2—Polygonal cells	<i>Sd</i> 67%	
Type 3—Small phagocytes	<i>Pl</i> 80%	
Red spherule cells	<i>Sp</i> 7-40%	Oxygen transport. Antibacterial activity from echinochrome A.
	<i>Sd</i> 8%	
	<i>Pl</i> 4.7%	
Colorless spherule cells	<i>Sp</i> 3.7-25%	Cytotoxicity, Clotting?
	<i>Sd</i> 6.5%	
	<i>Pl</i> 7.8%	
Vibratile cells	<i>Sp</i> 11.9-20%	Movement or agitation of coelomic fluid? Associated with clotting.
	<i>Sd</i> 18.5%	
	<i>Pl</i> 7.5%	

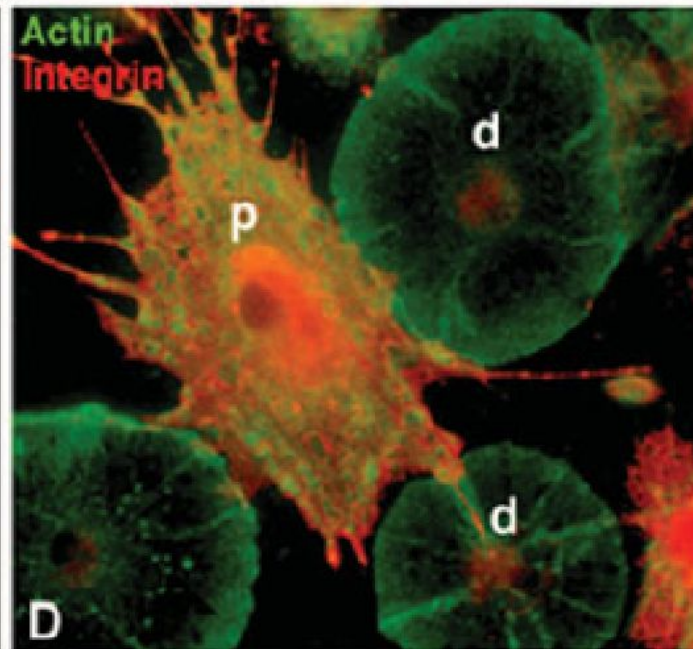
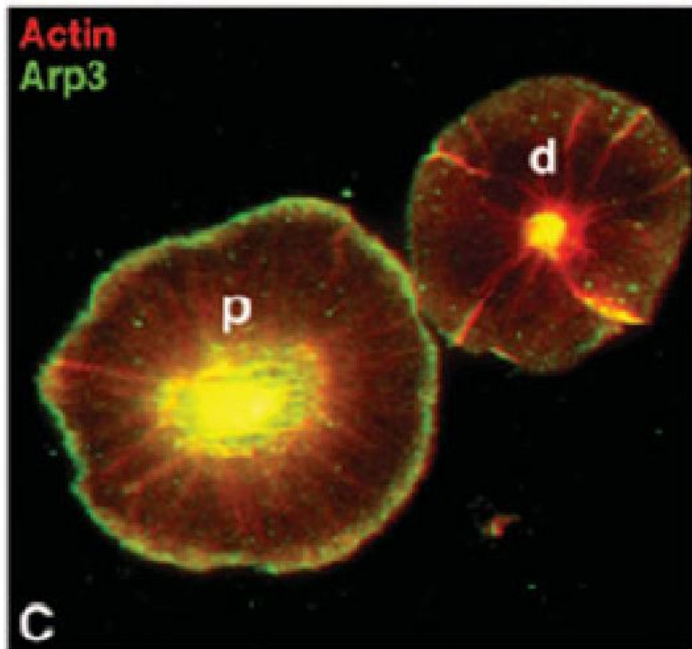
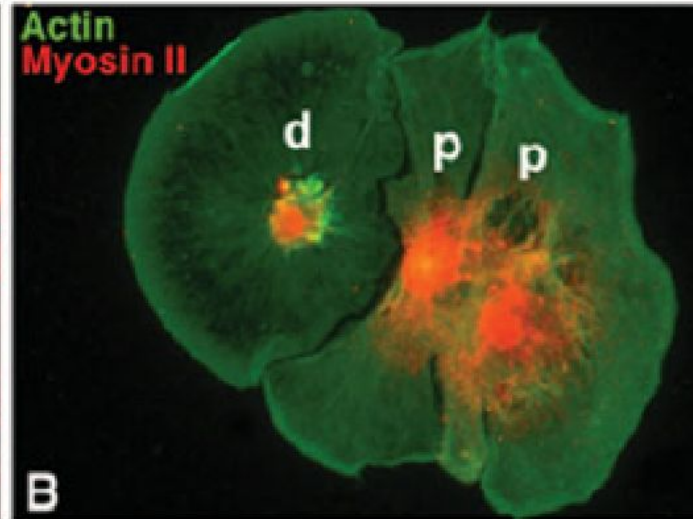
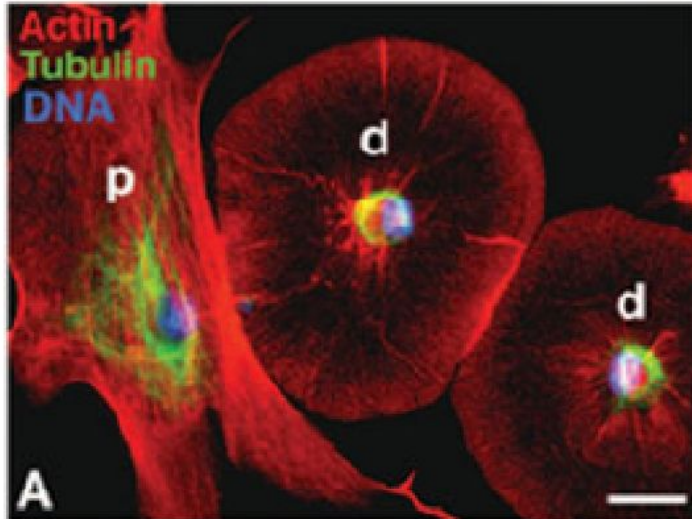
# Целомоциты морского ежа:



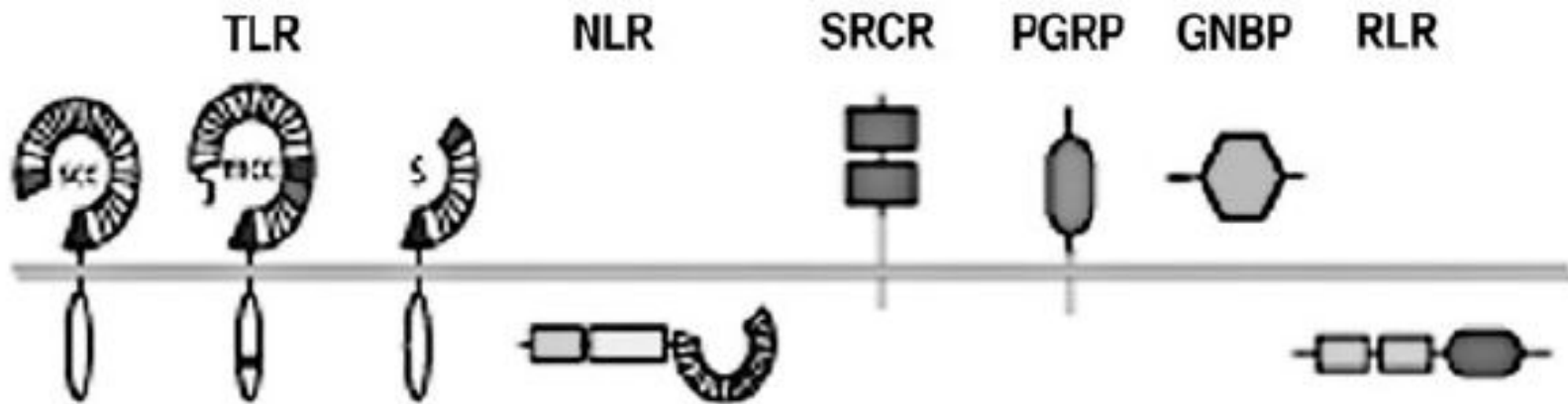


**Figure 2.** Coelomocytes from the sea urchin *S. droebachiensis*. Cells were withdrawn in anticoagulant and settled onto a glass coverslip. All cell types are shown and labeled with numbers. Large phagocytes; 1 = discoidal phagocyte; 2 = polygonal phagocyte; 3 = red spherule cell; 4 = colorless spherule cell; 5 = vibratile cell (the lower cell has lost the prominent flagellum seen in the upper cell). Inset; 6 = small phagocyte. Bar = 10 microns.

# Полигональные и дисковидные фагоциты отличаются организацией цитоскелета

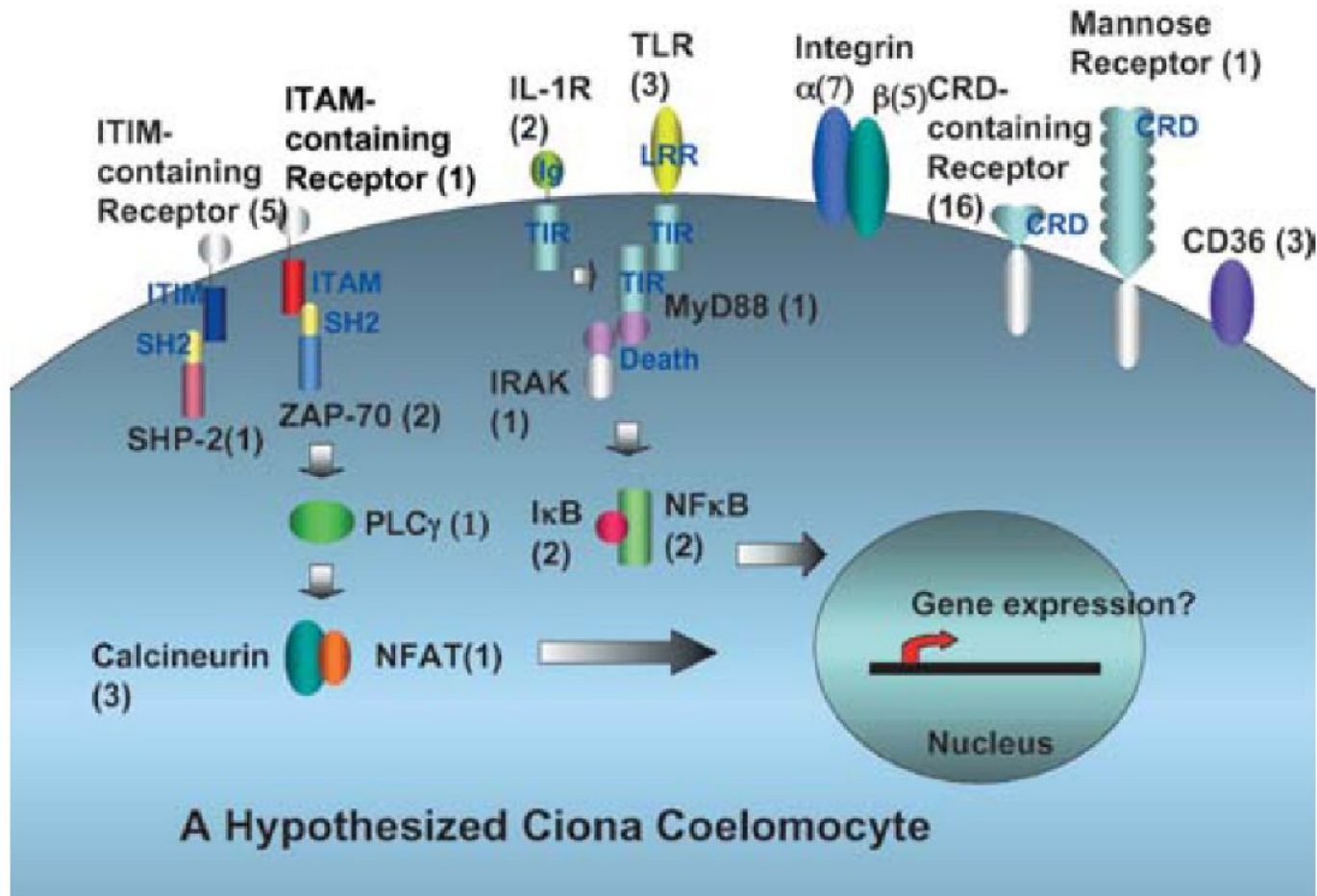


# Рецепторные белки иглокожих



<i>H.s.</i>	10(+1Ψ)	0	0	22	81(16)	6	0	3
<i>M.m.</i>	13	0	0	33	118(27)	4	0	3
<b><i>S.p.</i></b>	<b>214</b>	<b>3</b>	<b>5</b>	<b>&gt;200</b>	<b>1095(218)</b>	<b>5</b>	<b>3</b>	<b>12</b>
<i>D.m.</i>	1	8	0	0	14(7)	15	4	0
<i>C.e.</i>	0	1	0	0	3(1)	0	0	0

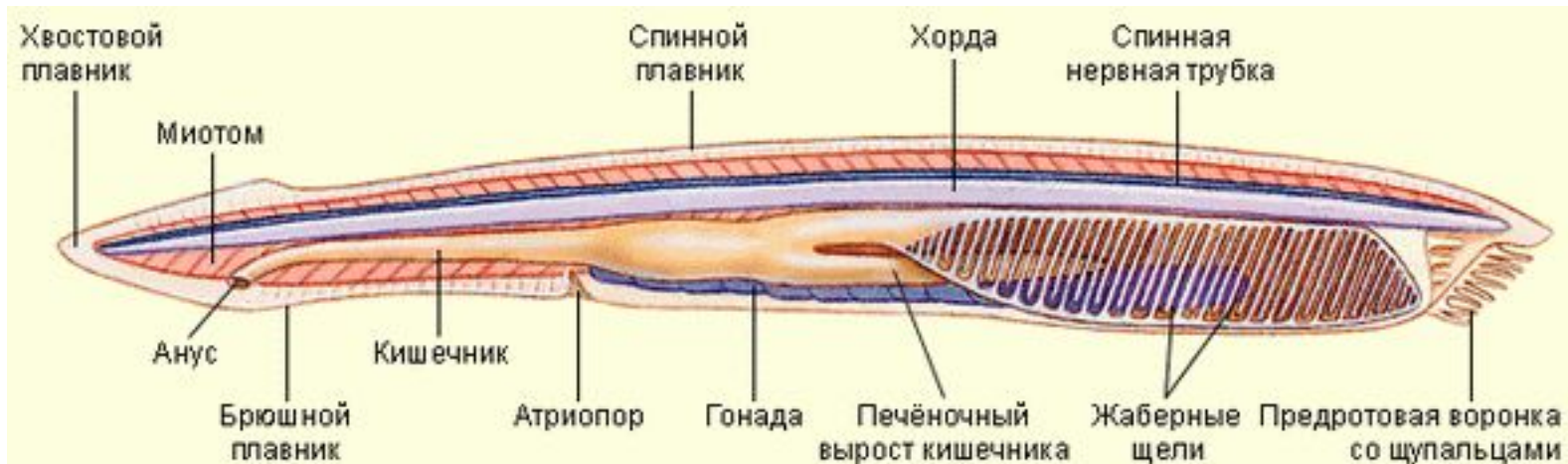
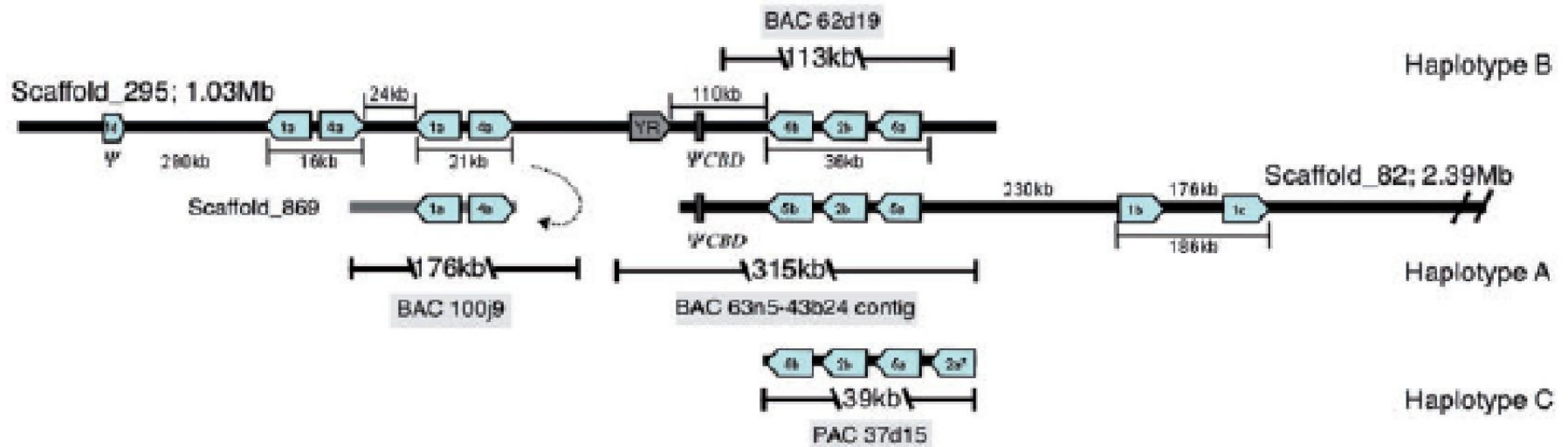
# Рецепторы целомоцита асцидии





# Гены VCBP у ланцетника

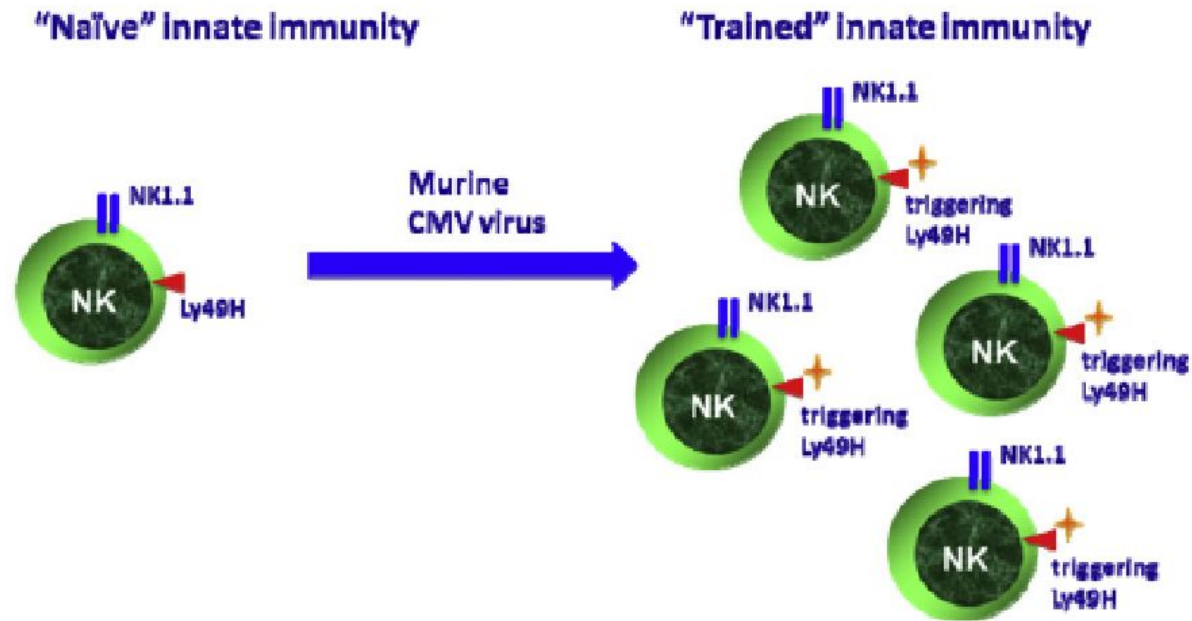
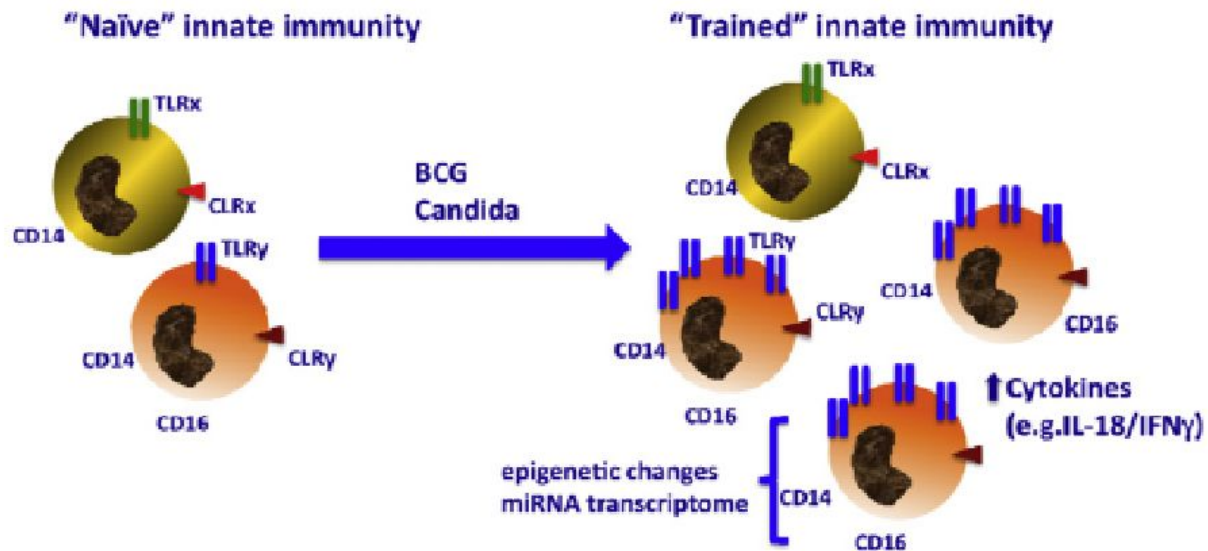
## a. Main chromosomal organization of amphioxus VCBP genes



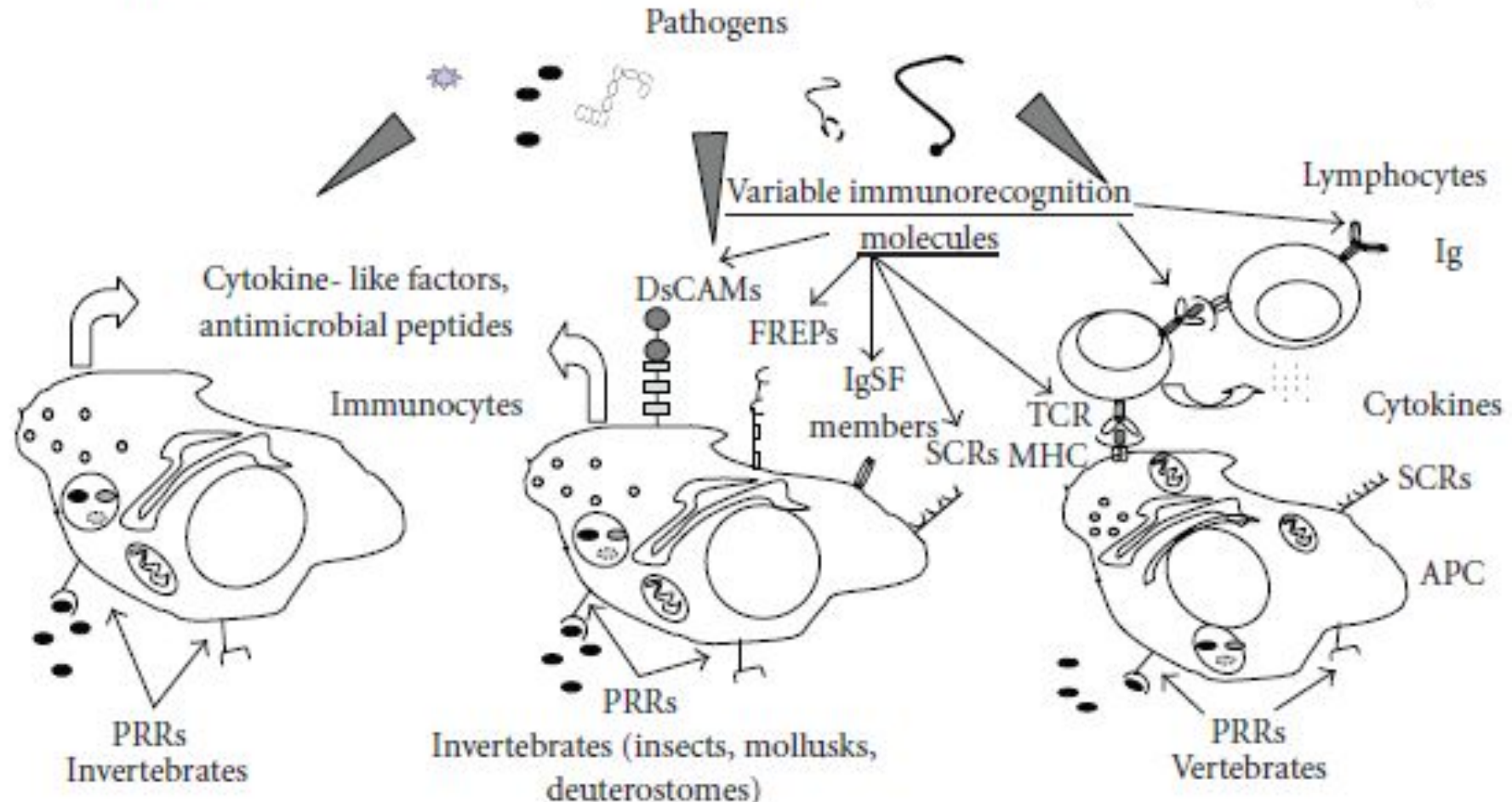
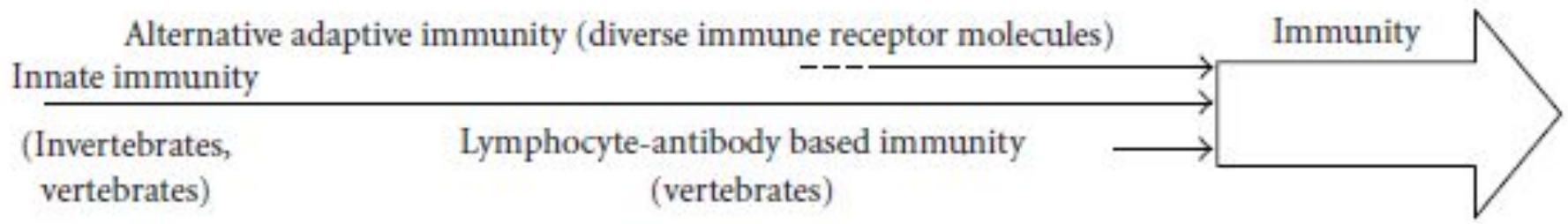
# «Тренированный» врождённый иммунитет – аналог адаптивного?

**Table 1. Selected Experimental Models in which Biological Activity Compatible with the Concept of Trained Innate Immunity Has Been Reported**

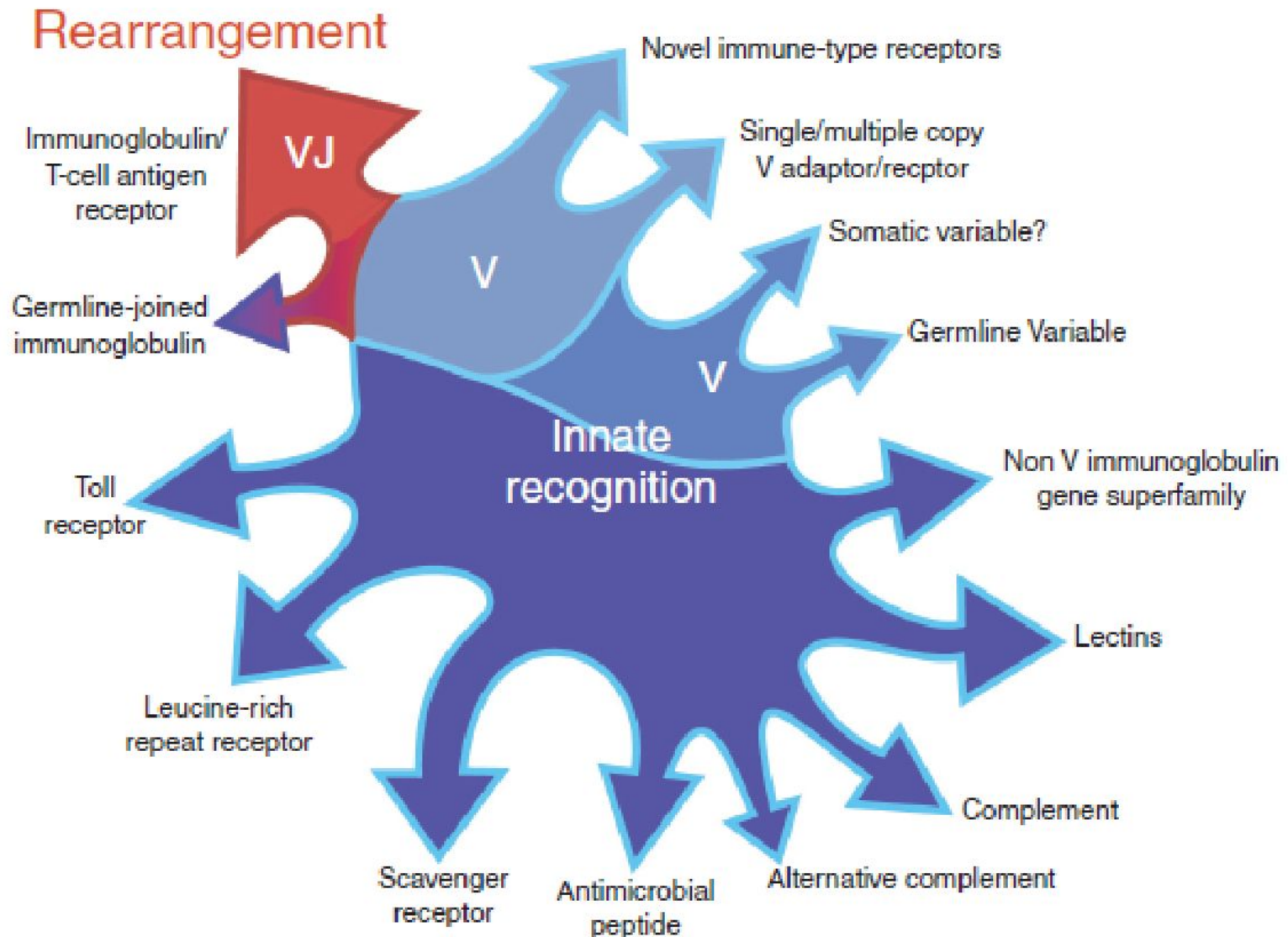
Organism	Experimental Model	Biological Effect	Specificity	References
<b>Plants – Systemic Acquired Resistance</b>				
Large variety of plants	Viruses, bacteria, fungi	Protection against reinfection	Variable	Durrant and Dong, 2004; Sticher et al., 1997
<b>Nonvertebrates</b>				
Mealworm beetle	LPS, or bacterial prechallenge	Protection against secondary infection	No	Moret and Siva-Jothy, 2003
<i>Drosophila</i>	<i>S. pneumoniae</i> prechallenge	Protection against <i>S. pneumoniae</i>	Uncertain	Pham et al., 2007
<i>Anopheles gambiae</i>	<i>Plasmodium</i> prechallenge	Protection against <i>Plasmodium</i>	No	Rodrigues et al., 2010
Sponges	Transplantation	Rejection	Yes	Hildemann et al., 1979
Corals	Transplantation	Rejection	Yes	Hildemann et al., 1977
<b>Vertebrates</b>				
Mice	BCG	Protection against candidiasis	No	Van 't Wout et al., 1992
Mice	<i>Candida</i> vaccination	T/B cell-independent protection	No	Bistoni et al., 1986, 1988
Mice	Murine CMV infection	NK-dependent protection	No	Sun et al., 2009
Humans	BCG	Nonspecific protection to secondary infections	No	Garly et al., 2003

**A****B**

# Граница между врожденным и адаптивным иммунитетом



# Направления эволюции иммунных рецепторов



- Следующая лекция 26 марта – о трансплантационном иммунитете и возникновении адаптивного иммунитета