Эволюционная иммунология

Лекция 5 «Иммунитет дрозофилы»

Шилов Е.С.

12 марта 2018

Иммуноциты дрозофилы

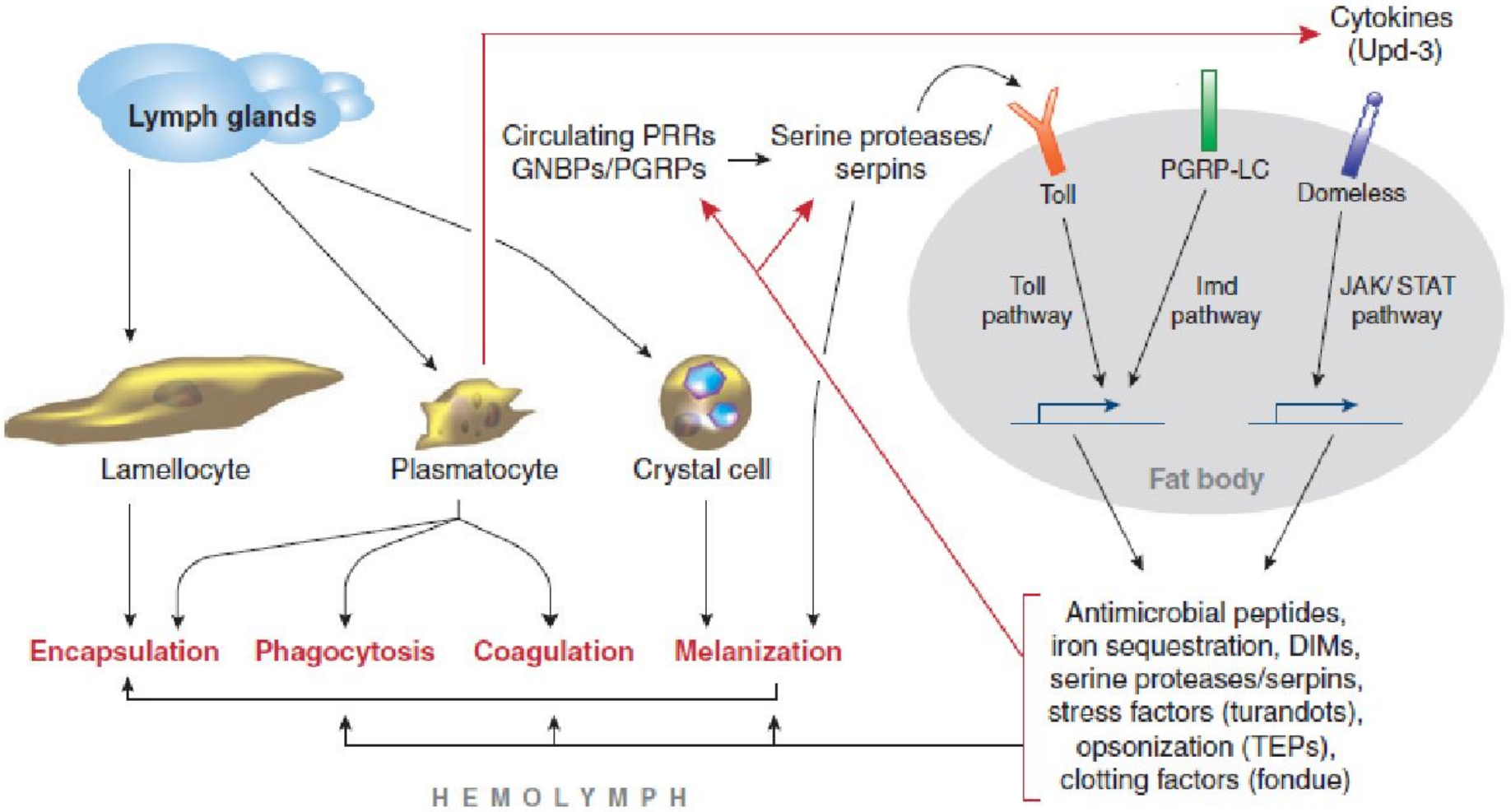
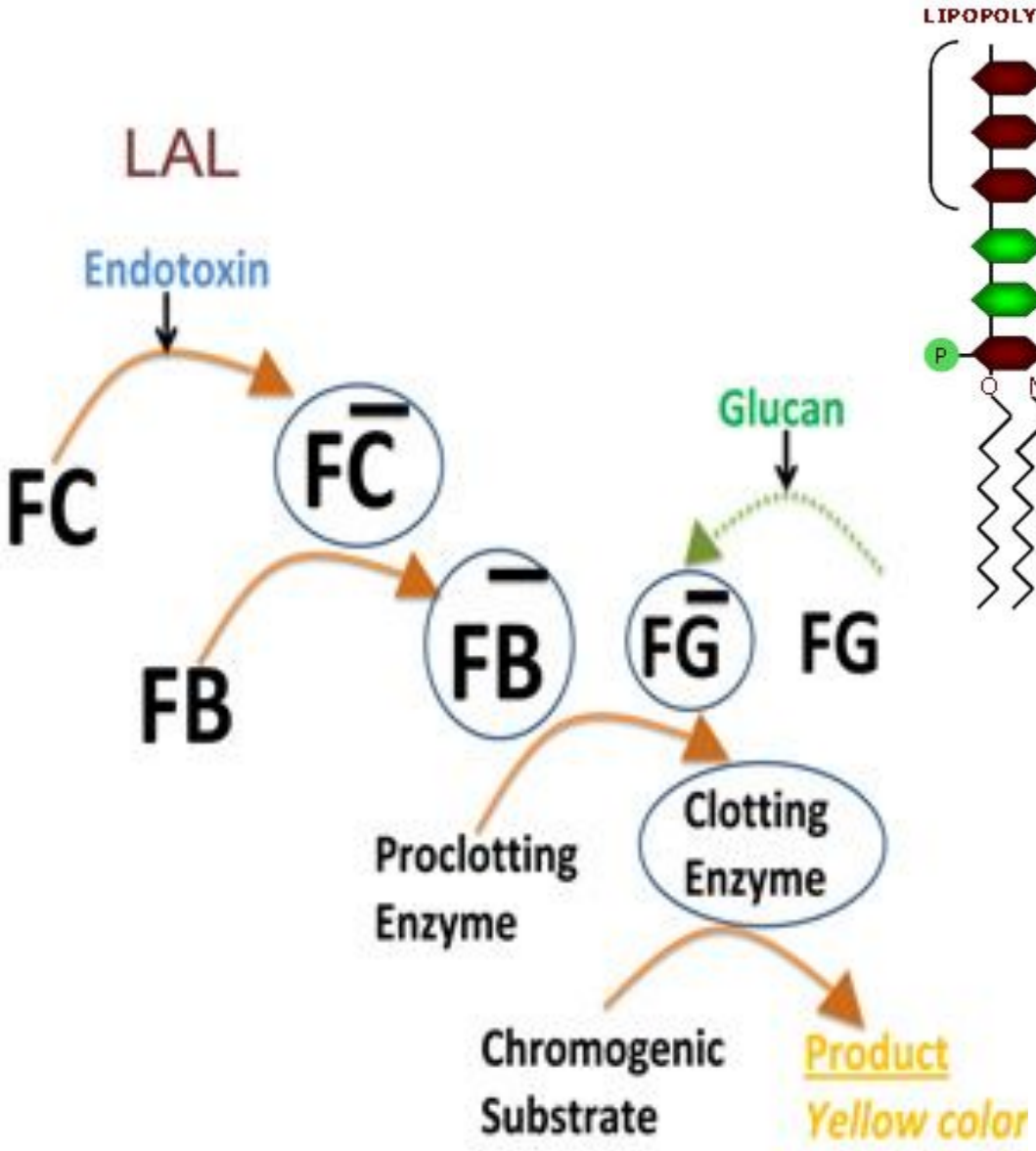
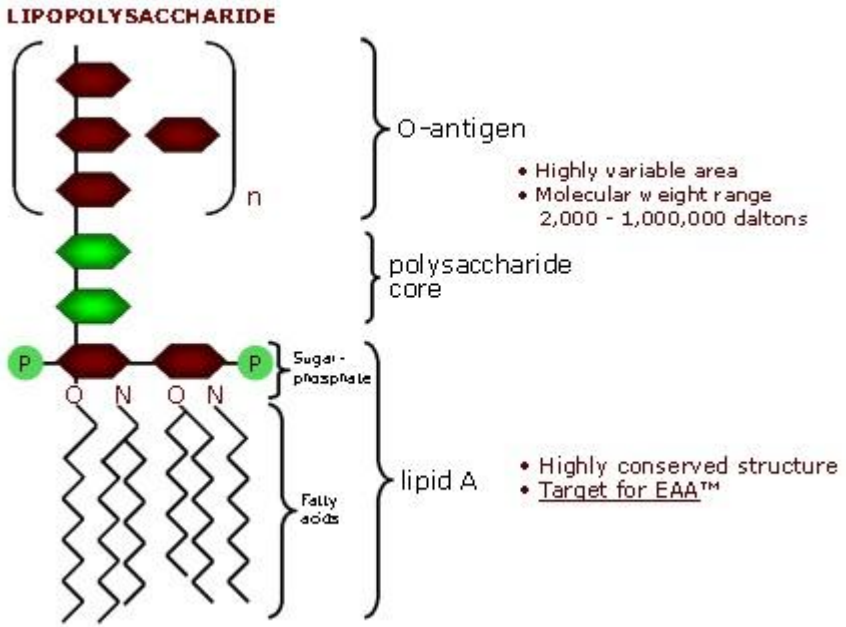


Figure 1

Schematic overview of *Drosophila* host defense. Detection of microbial pathogens elicits a large array of interconnected and synergistic defense modules in immune-responsive tissues.

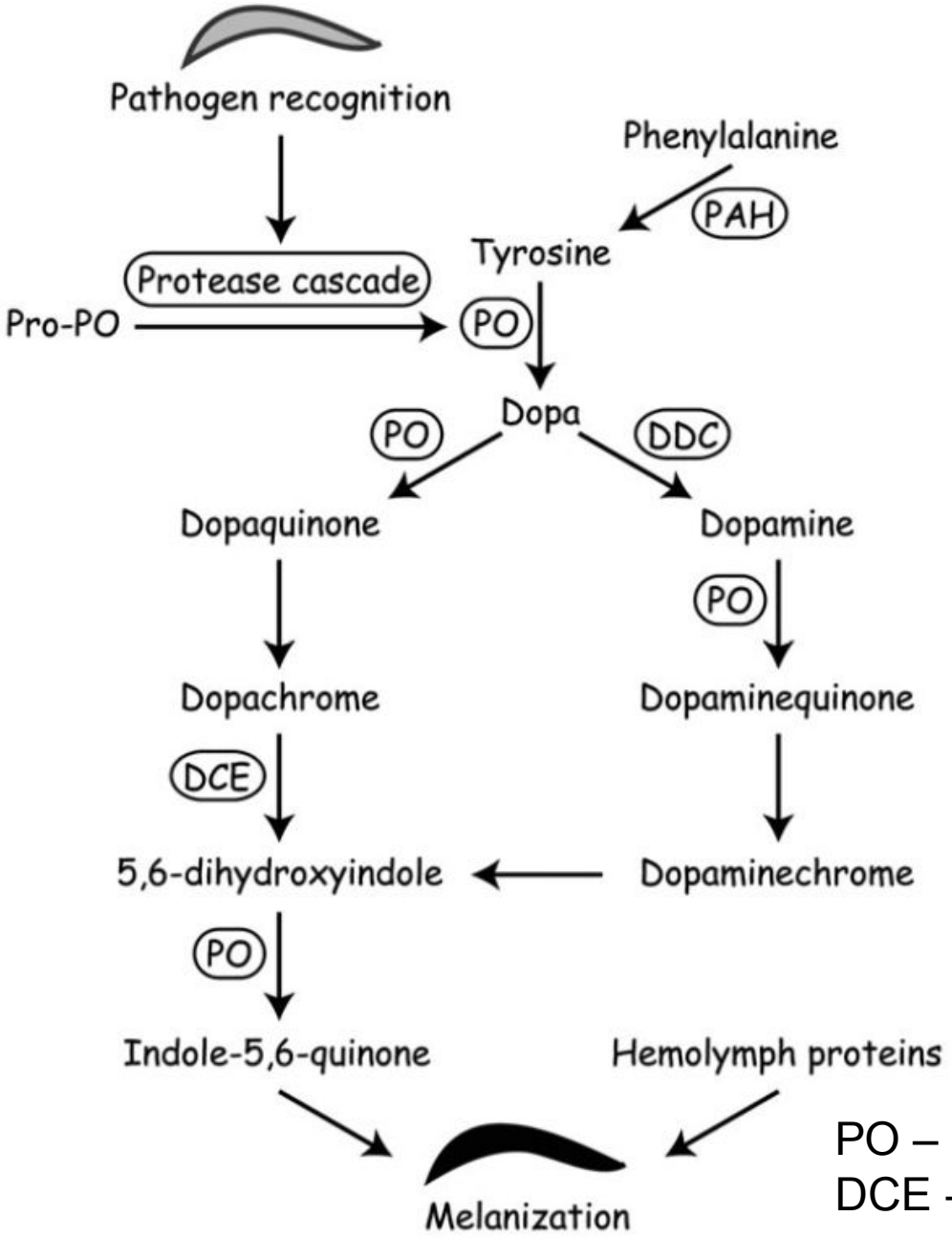
Лизат амёбоцитов мечехвоста – система детекции ЛПС





Limulus polyphemus

СИНТЕЗ МЕЛАНИНА



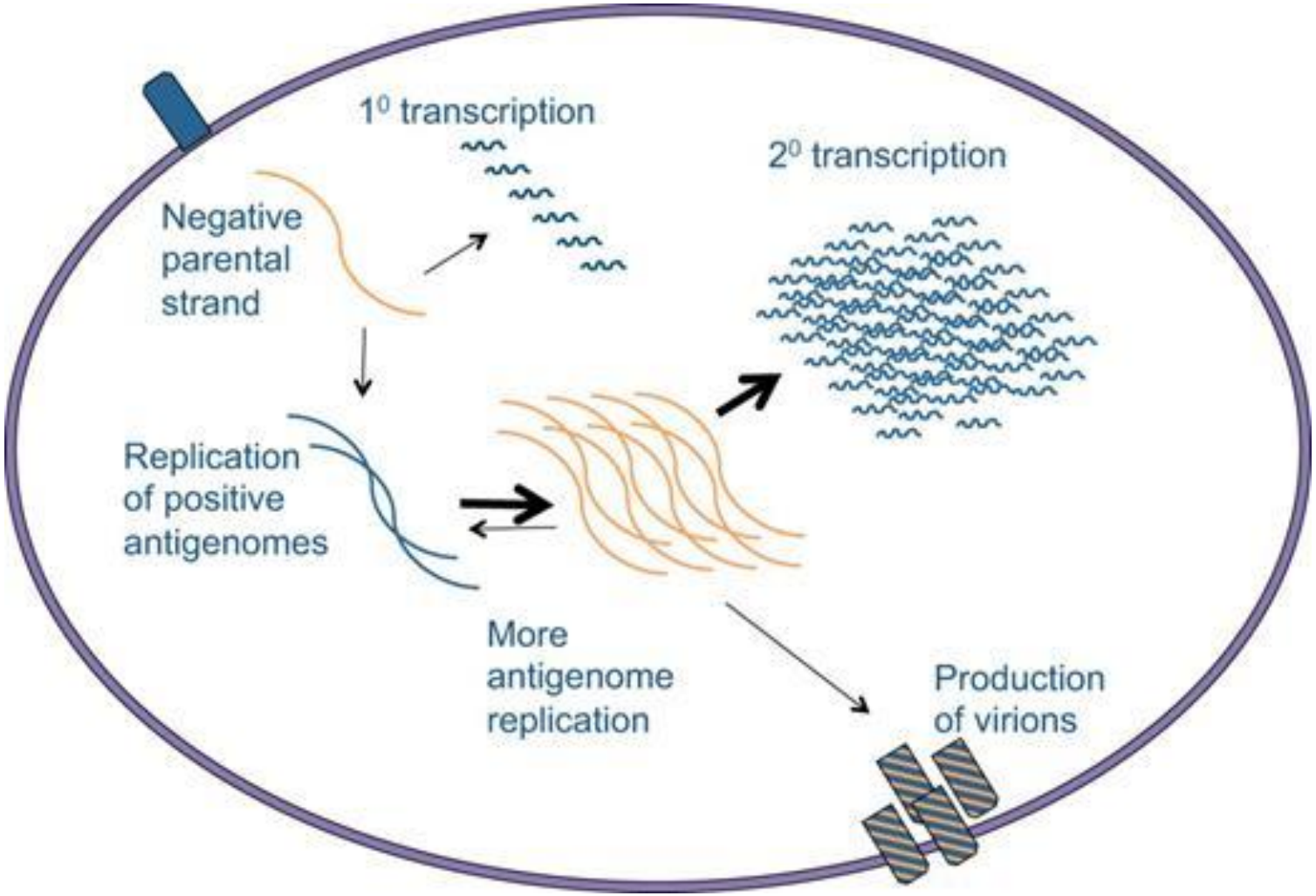
PO – фенолоксидаза
DCE - допахромконвертаза

Вирусы членистоногих обычно имеют РНК-геном

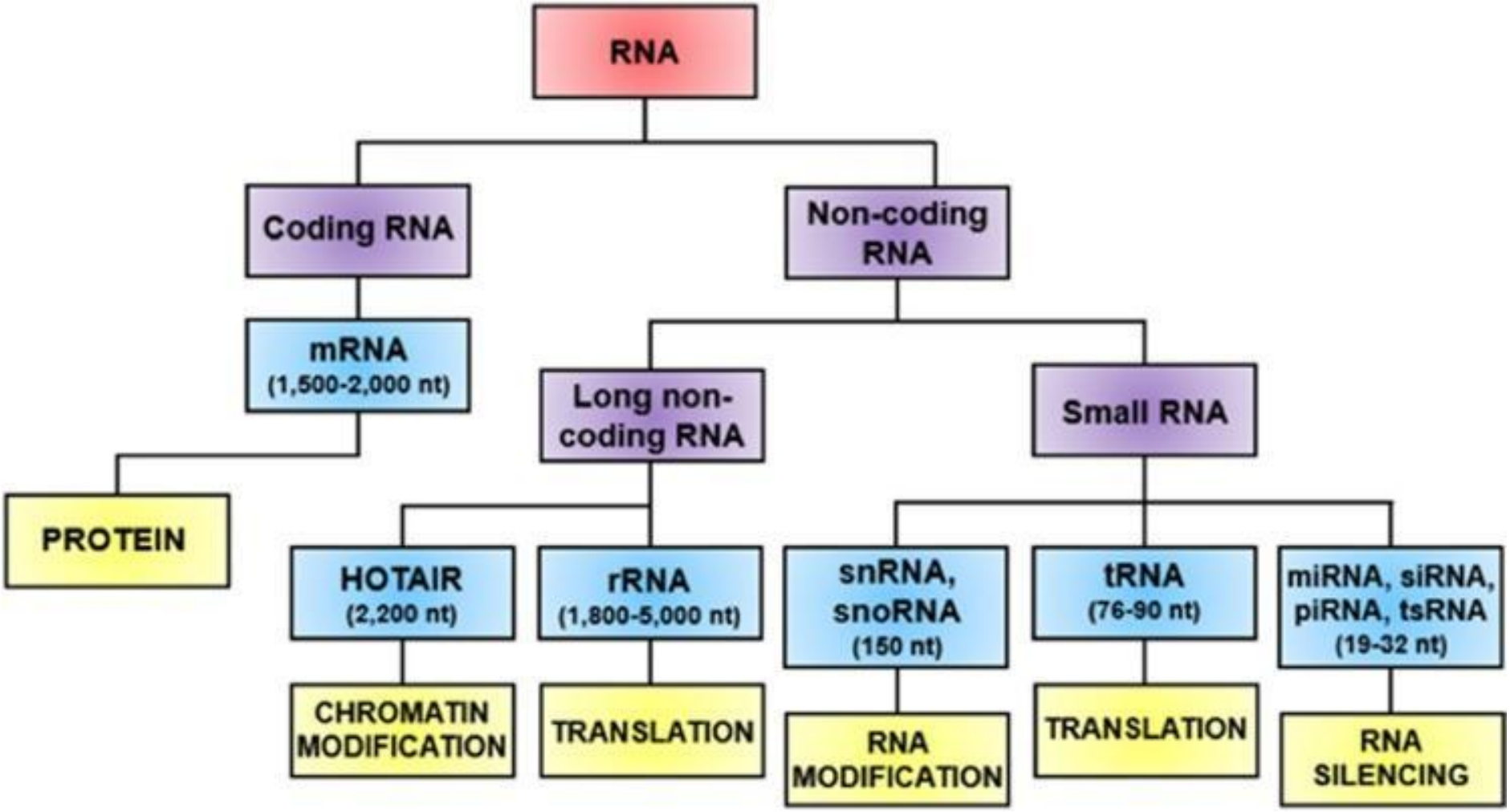
Virus-derived small RNA profiles of arthropod viruses. Modified from (van Mierlo et al., 2011).

Virus	Family	Host for study	Genome
RNA viruses			
Drosophila A virus	Tetraviridae	Drosophila[b]	(+)ssRNA
Drosophila C virus	Dicistroviridae	Drosophila[b]	(+)ssRNA
Homalodisca coagulata virus-1	Dicistroviridae	Homalodisca vitripennis[a]	(+)ssRNA
Noravirus	Unassigned	Drosophila[b]	(+)ssRNA
Sindbis virus	Alphaviridae	Aedes aegypti[a]	(+)ssRNA
Mosquito nodavirus	Nodaviridae	Aedes aegypti[a]	(+)ssRNA
West Nile virus	Flaviviridae	Culex pipiens quinquefasciatus[a]	(+)ssRNA
Semliki Forest virus	Alphaviridae	Aedes albopictus[b] and Aedes aegypti[b]	(+)ssRNA
Dengue virus –Serotype 2	Flaviviridae	Aedes aegypti[a,b]	(+)ssRNA
Cell fusing agent virus	Flaviviridae	Aedes aegypti[b]	(+)ssRNA
Flock house virus	Nodaviridae	Drosophila[a,b]	Bipartite (+) RNA
American nodavirus	Nodaviridae	Drosophila[b]	Bipartite (+) RNA
Vesicular stomatitis virus	Rhabdoviridae	Drosophila[a,b]	(−) ssRNA
Rift Valley fever virus	Bunyaviridae	Drosophila[b]	(−)ssRNA
Drosophila totivirus	Totiviridae	Drosophila[b]	dsRNA
Drosophila X virus	Birnaviridae	Drosophila[b]	Bipartite dsRNA
Drosophila birnavirus	Birnaviridae	Drosophila[b]	Bipartite dsRNA
Homalodisca vitripennis reovirus	Reoviridae	Homalodisca vitripennis[a]	dsRNA
Mosquito X virus	Unassigned	Culex pipiens molestus[a], Culex tritaeniorhynchus[a], Aedes sinensis[a]	Bipartite dsRNA
DNA viruses			
Helicoverpa armigera single nucleopolyhedrovirus	Baculoviridae	Helicoverpa armigera[a]	dsDNA
Vaccinia virus	Poxviridae	Drosophila[b]	dsDNA
Invertebrate iridescent virus 6	Iridoviridae	Drosophila[a,b]	dsDNA

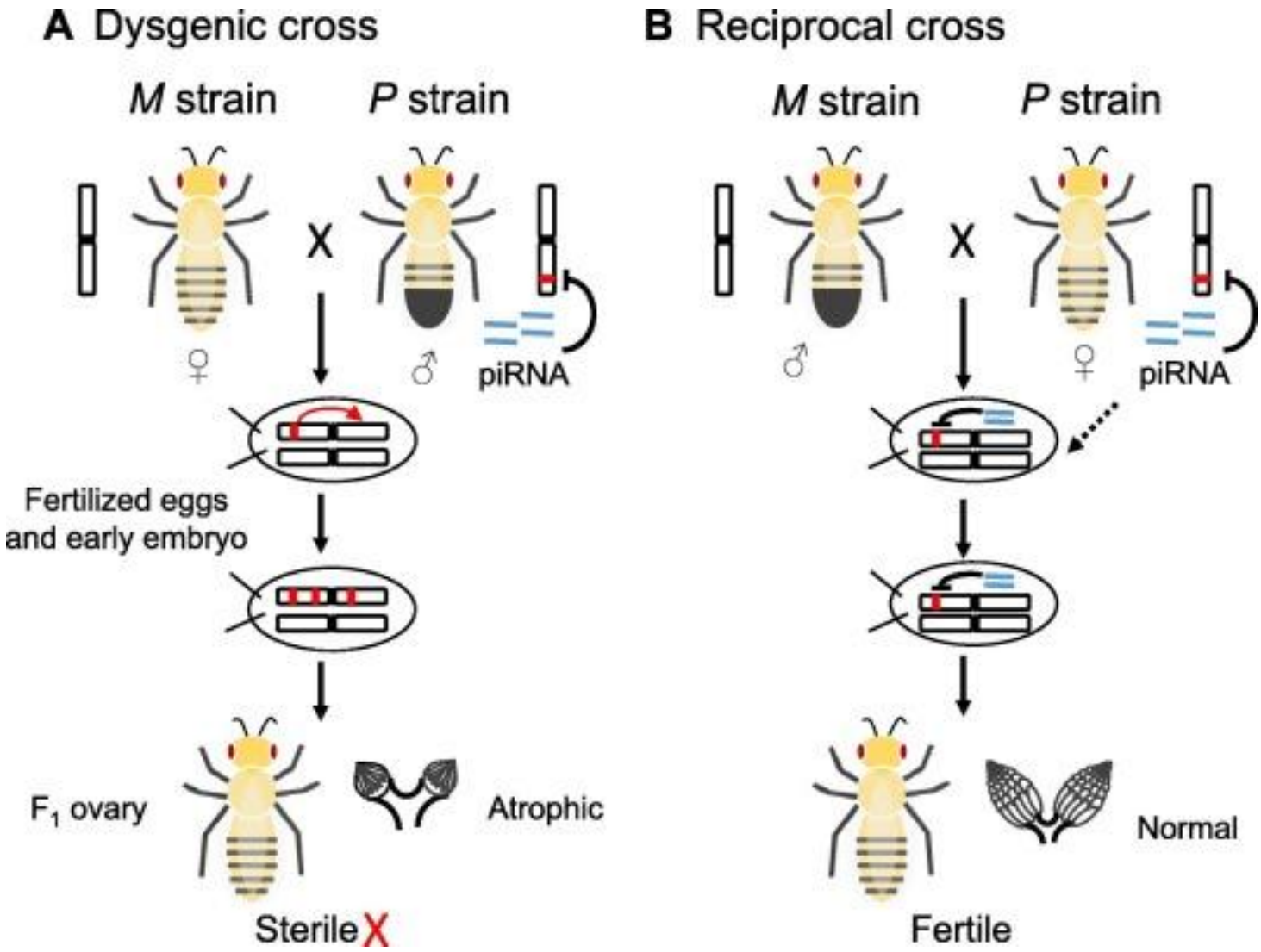
Цикл репликации вируса дрозофилы DmelSV



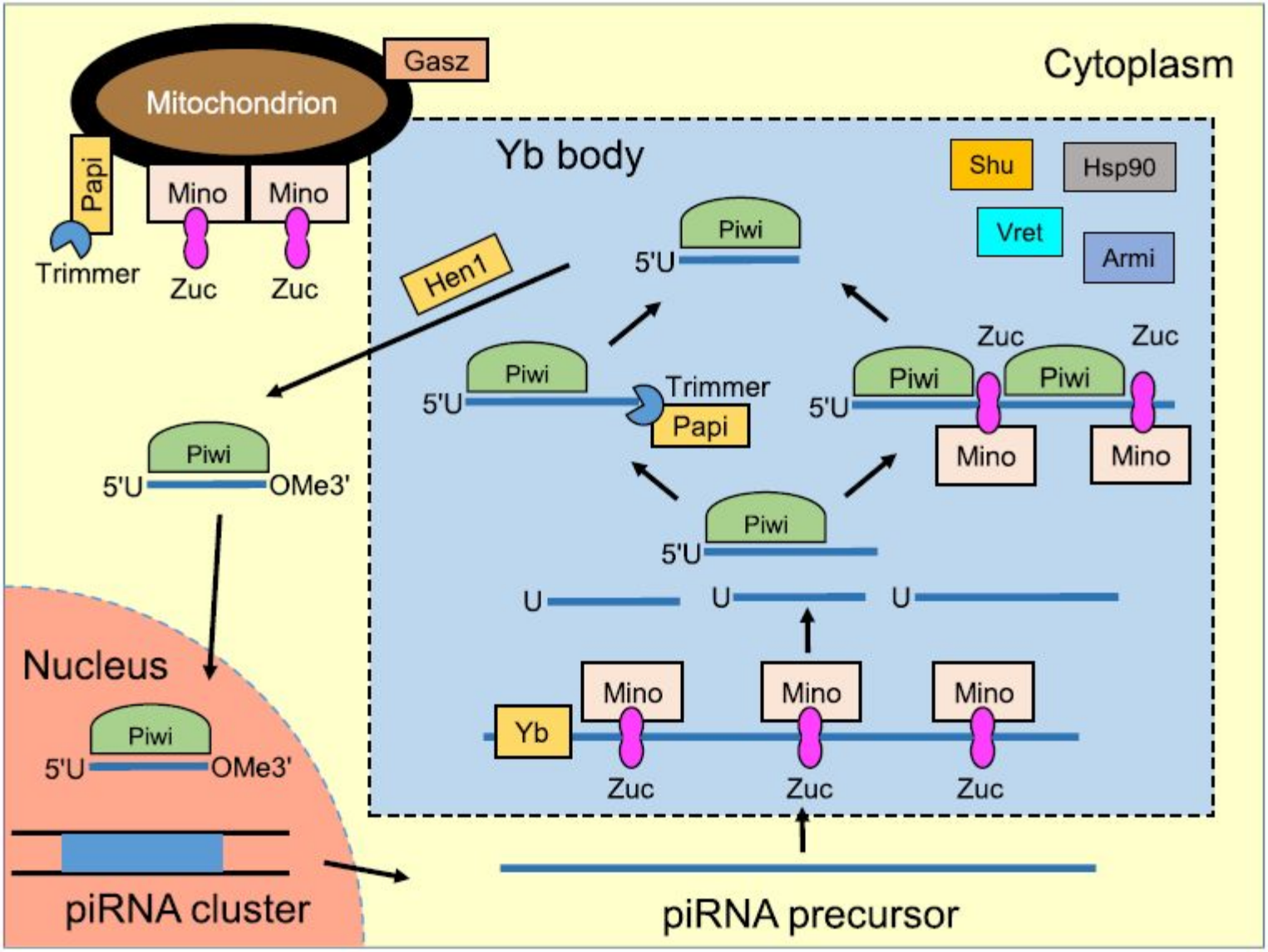
Классификация видов РНК эукариот



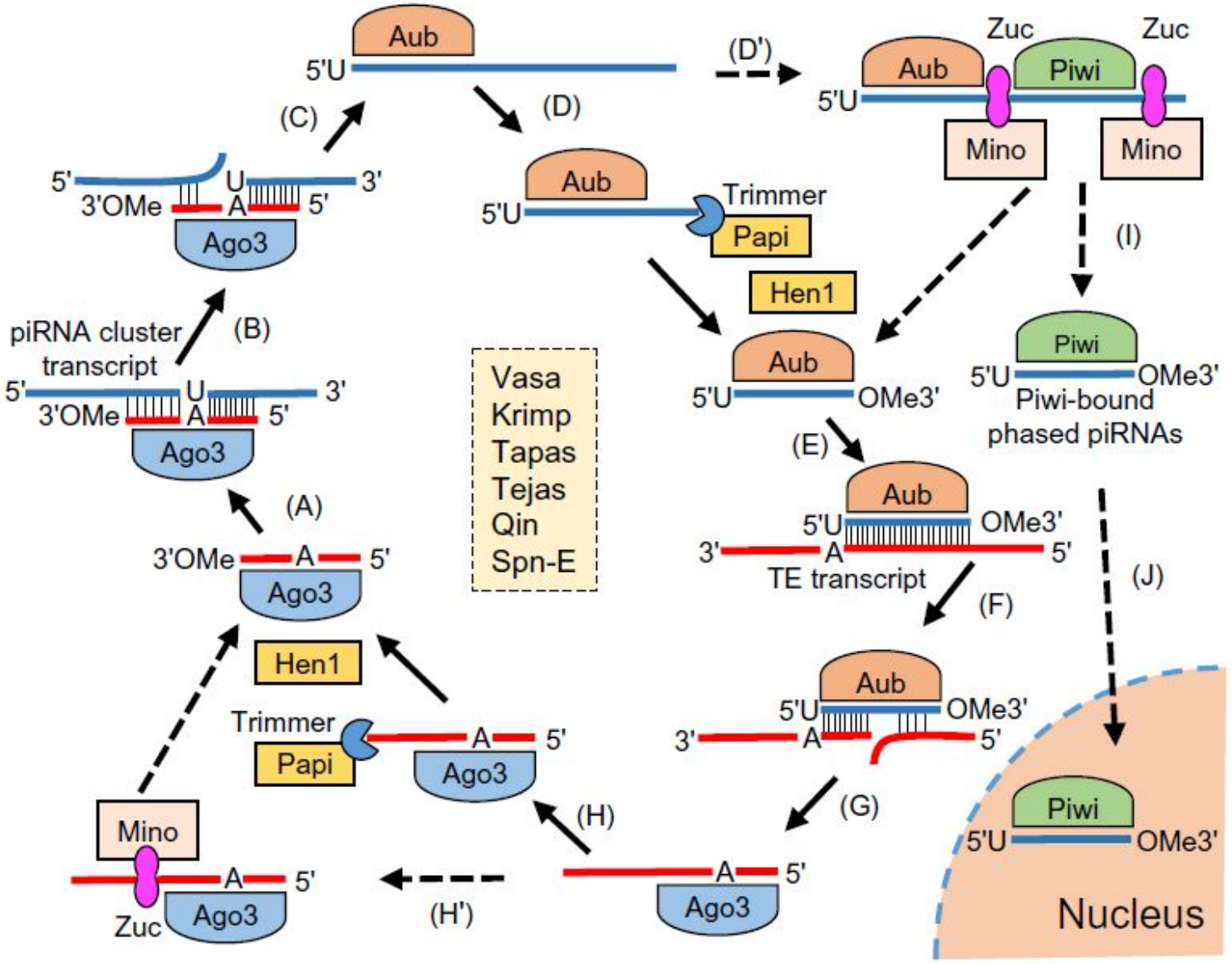
Гибридный дисгенез у дрозофилы



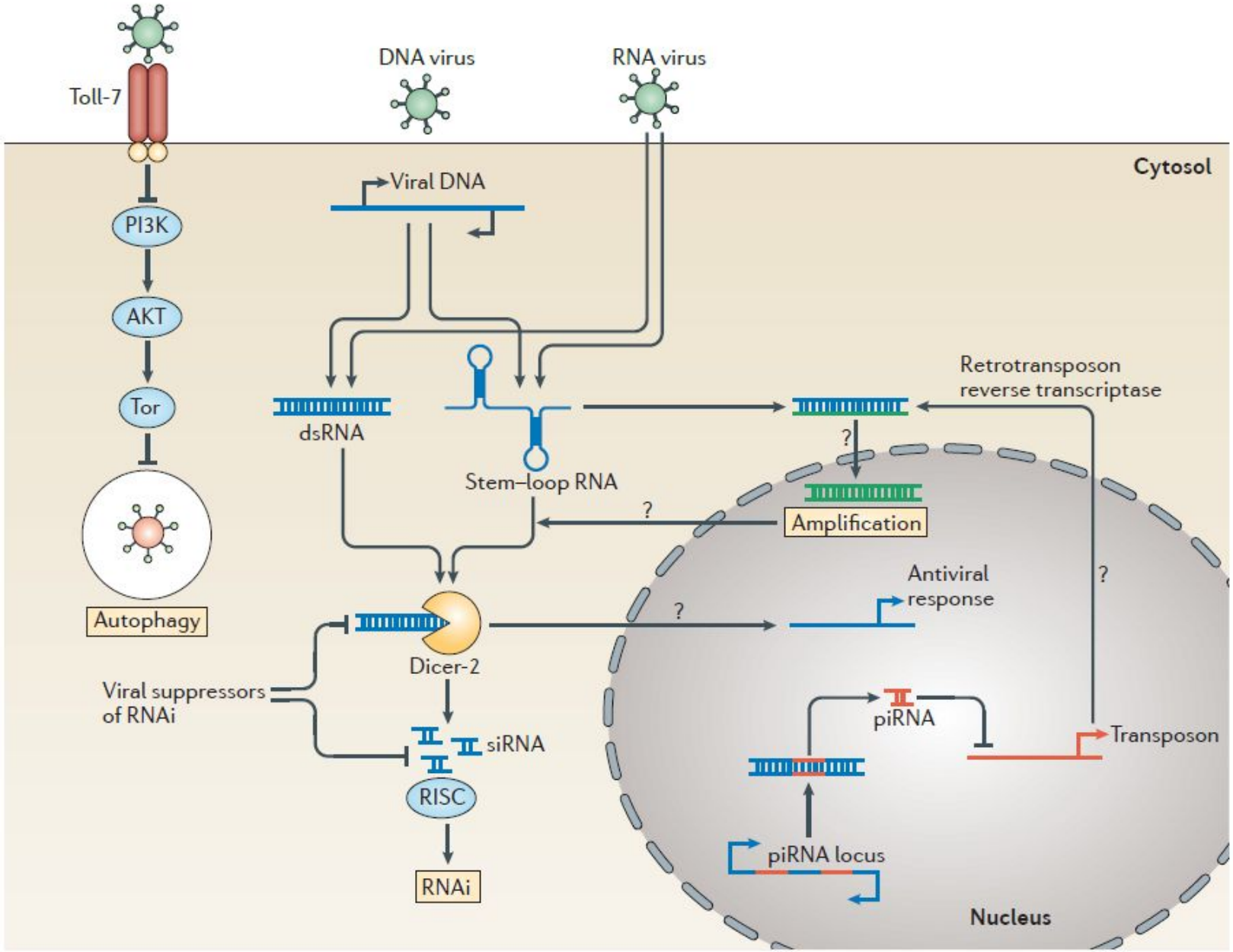
Первичный процессинг пиРНК



«Игра в пинг-понг» и амплификация пиРНК



Противовирусный иммунитет дрозофилы



Принципиальная схема РНК-интерференции у дрозофилы

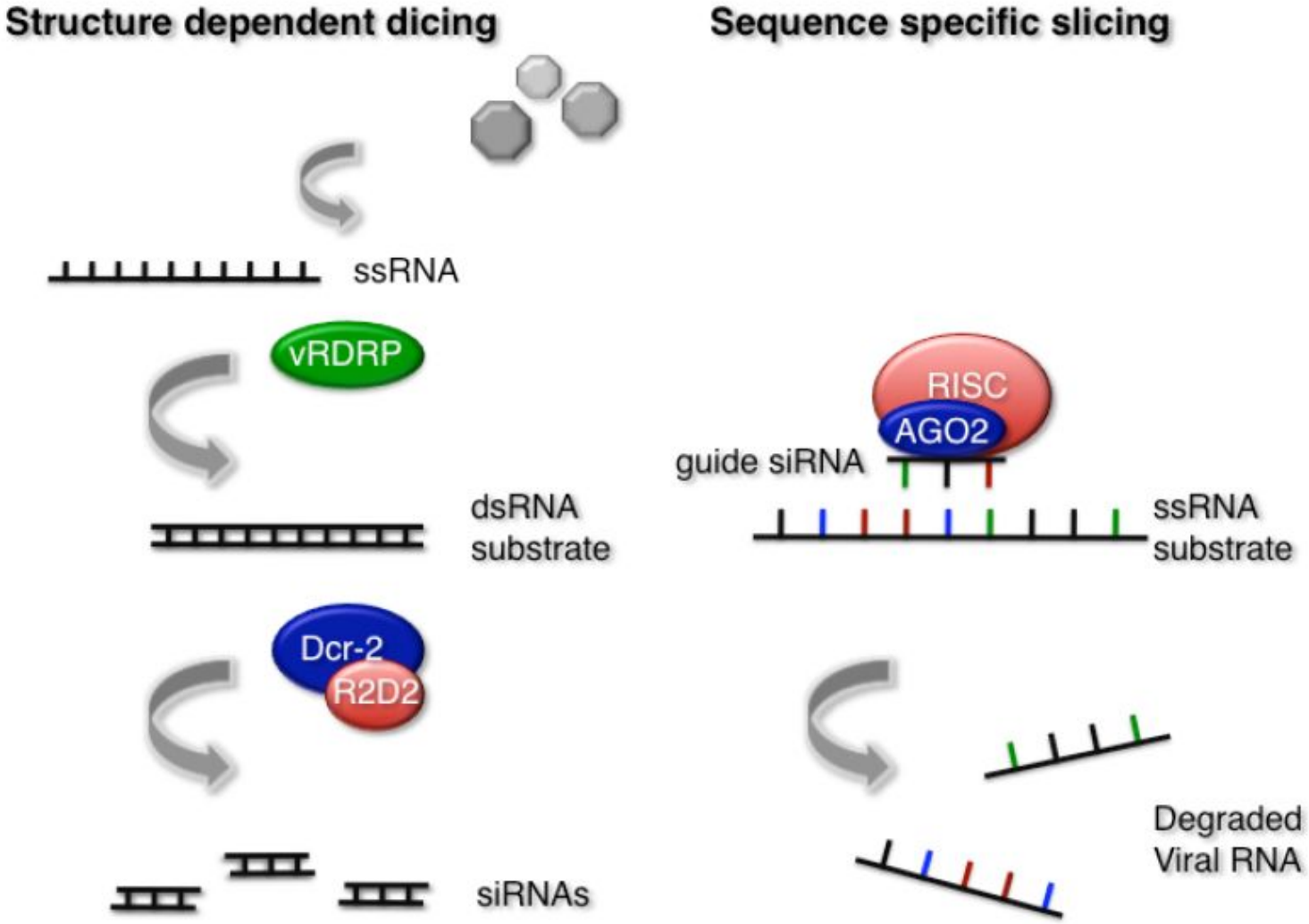
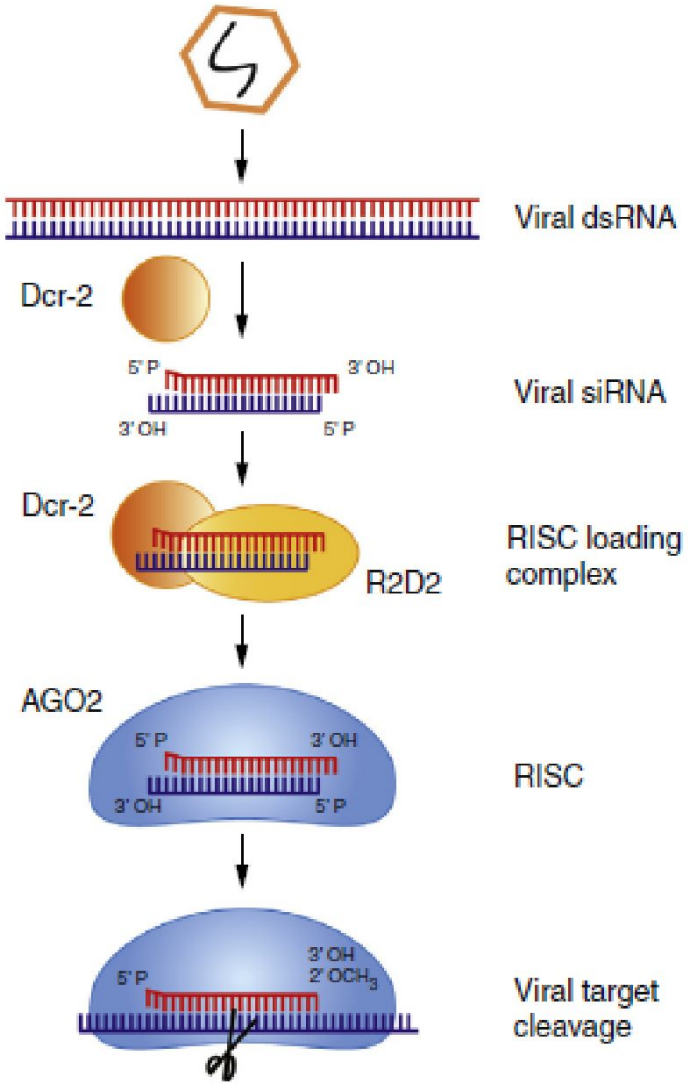
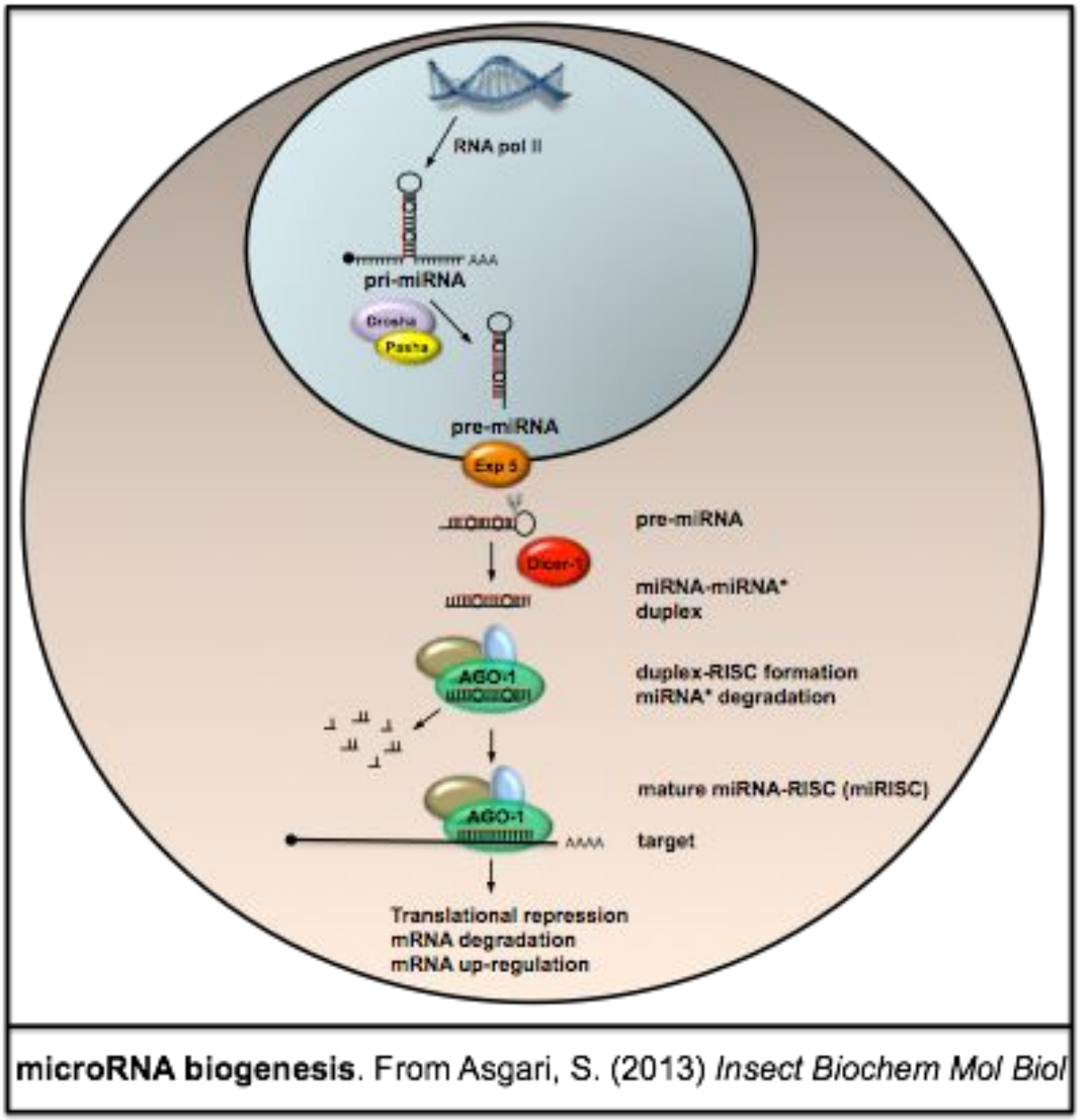


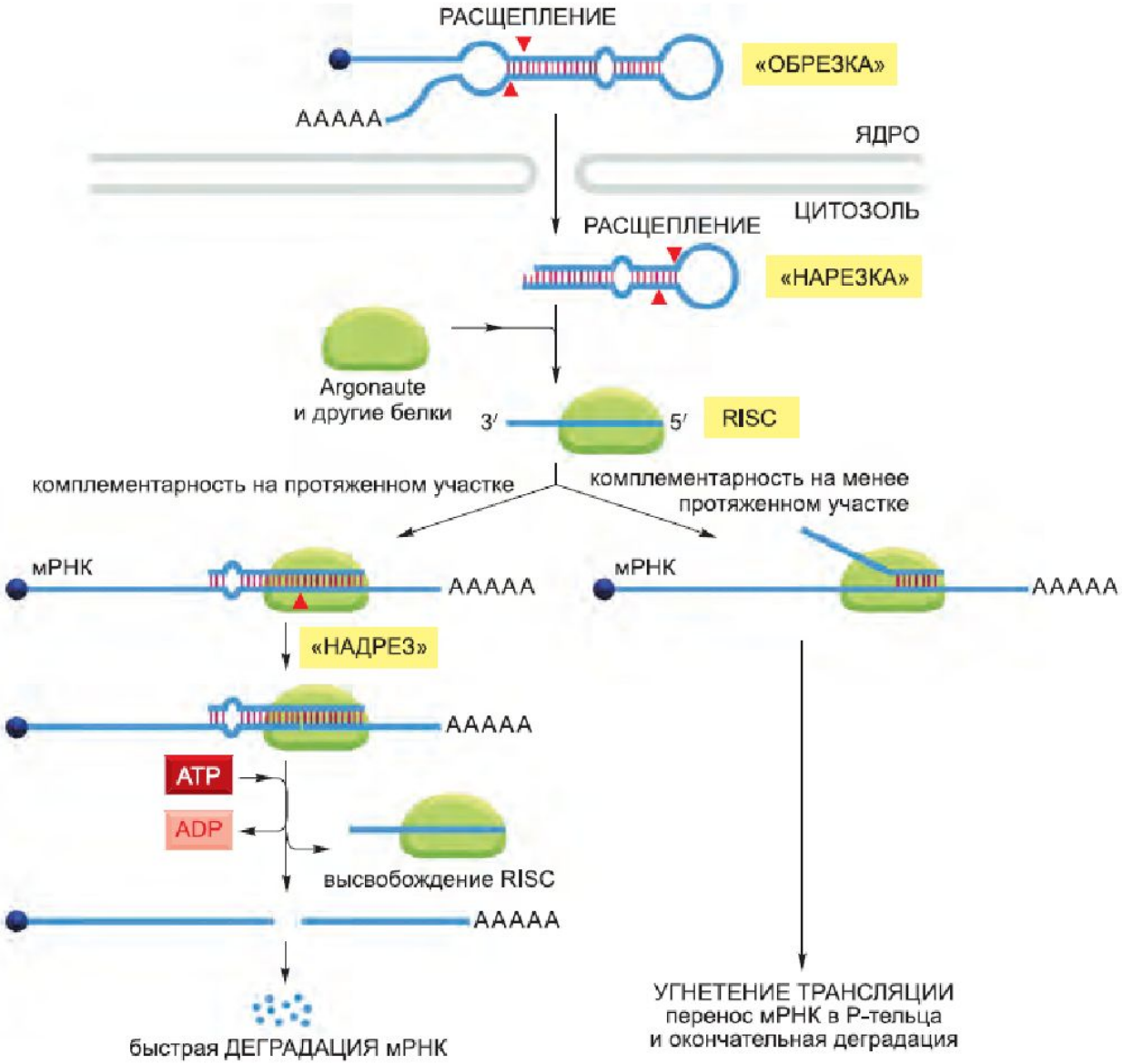
Схема образования miRNA и siRNA у дрозофилы

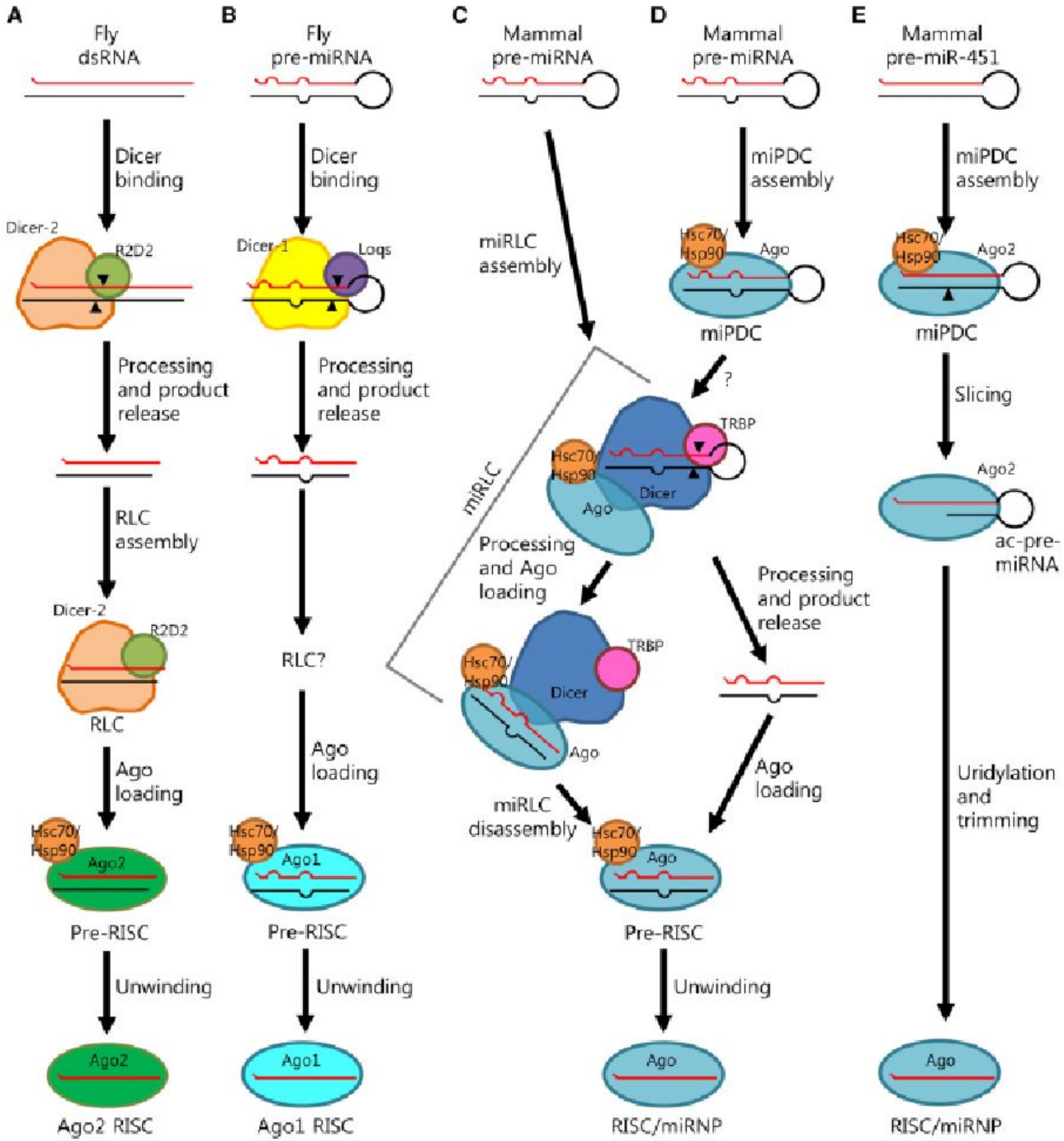




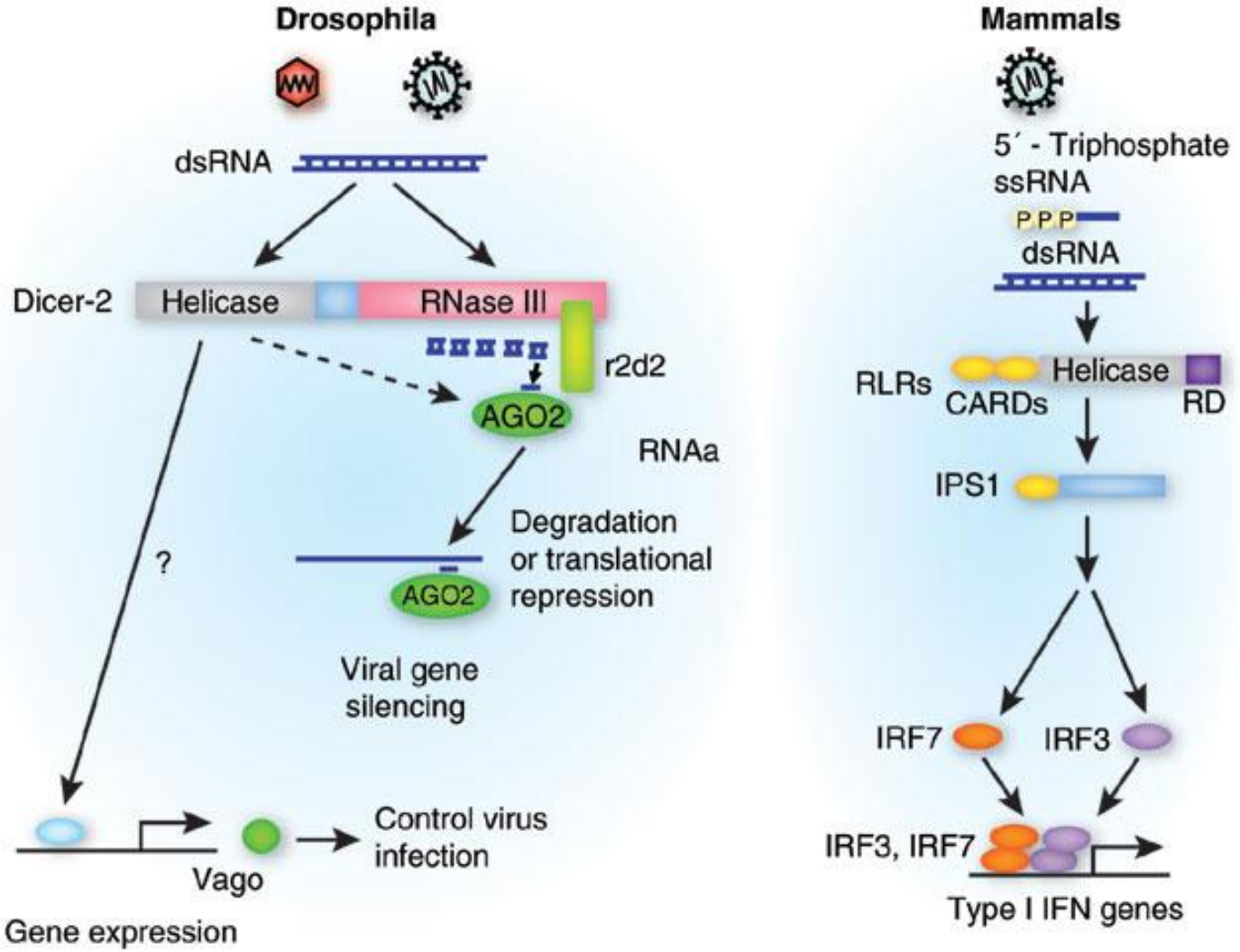
microRNA biogenesis. From Asgari, S. (2013) *Insect Biochem Mol Biol*

По такому же механизму (с помощью миРНК) регулируются и собственные гены эукариот

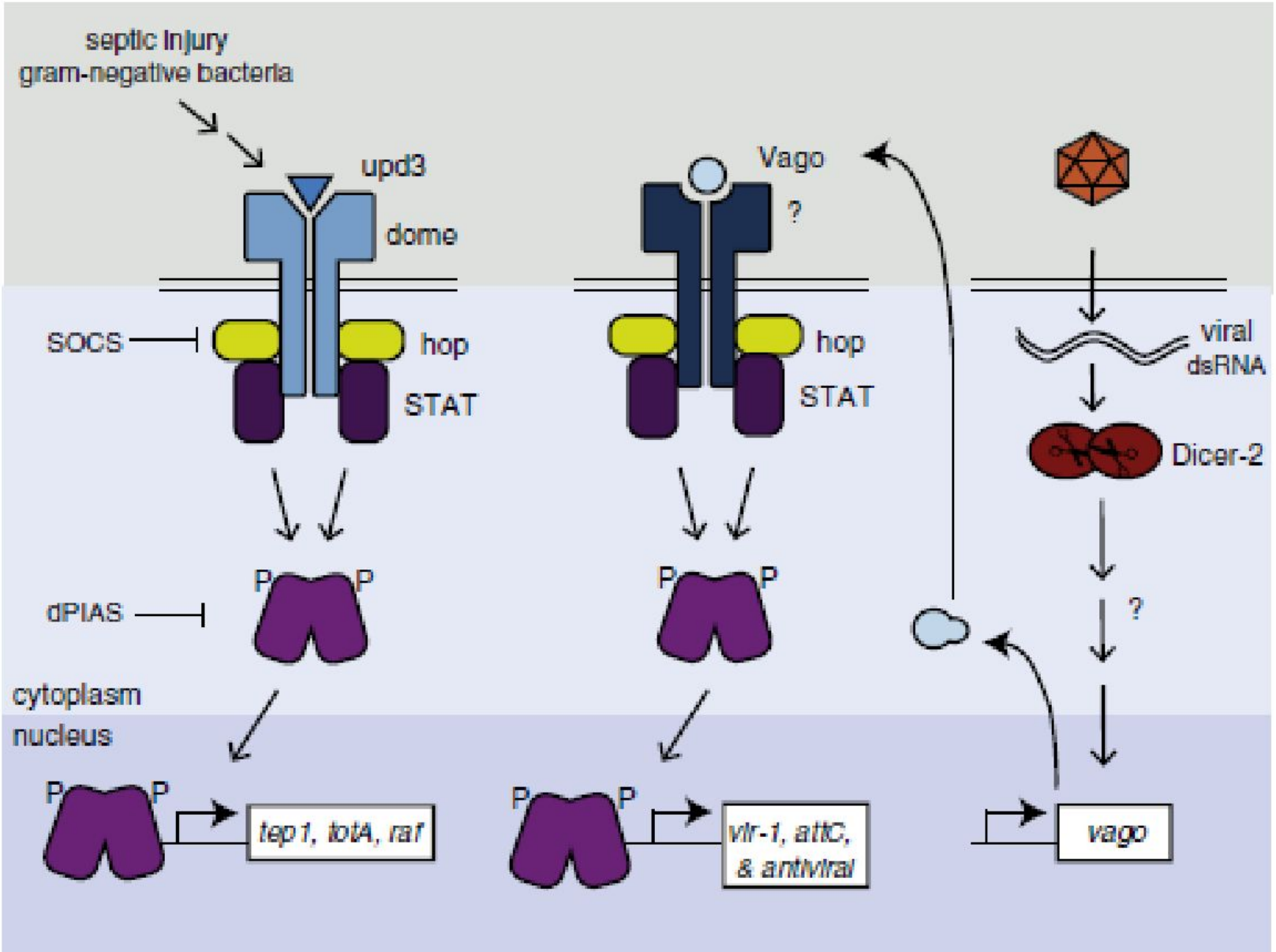



A
Fly
dsRNA
Dicer
binding
Dicer-2
R2D2
Processing
and product
release
RLC
assembly
Dicer-2
R2D2
RLC
Ago
loading
Hsc70/
Hsp90
Ago2
Pre-RISC
Unwinding
Ago2
Ago2 RISC
B
Fly
pre-miRNA
Dicer
binding
Dicer-1
Loqs
Processing
and product
release
RLC?
Ago
loading
Hsc70/
Hsp90
Ago1
Pre-RISC
Unwinding
Ago1
Ago1 RISC
C
Mammal
pre-miRNA
miRLC
assembly
miRLC
?
TRBP
Hsc70/
Hsp90
Dicer
Ago
Processing
and Ago
loading
TRBP
Hsc70/
Hsp90
Dicer
Ago
miRLC
disassembly
Hsc70/
Hsp90
Ago
Pre-RISC
Unwinding
Ago
RISC/miRNP
D
Mammal
pre-miRNA
miPDC
assembly
Hsc70/
Hsp90
Ago
miPDC
Processing
and product
release
Ago
loading
E
Mammal
pre-miR-451
miPDC
assembly
Hsc70/
Hsp90
Ago2
miPDC
Slicing
Ago2
ac-pre-
miRNA
Uridylation
and
trimming
Ago
RISC/miRNP

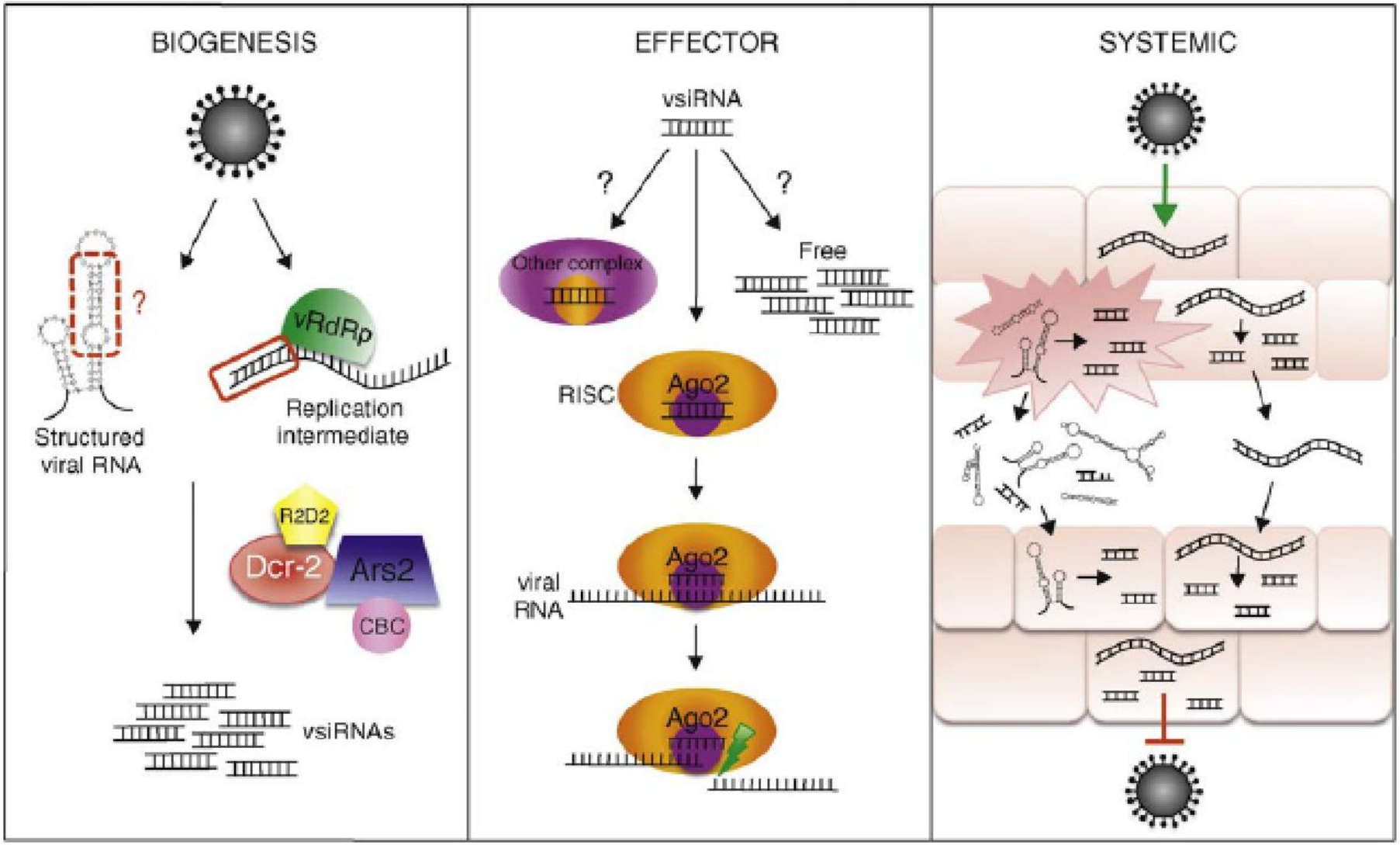
Различия между RNAi дрозофилы и RLR-сигналингом млекопитающих



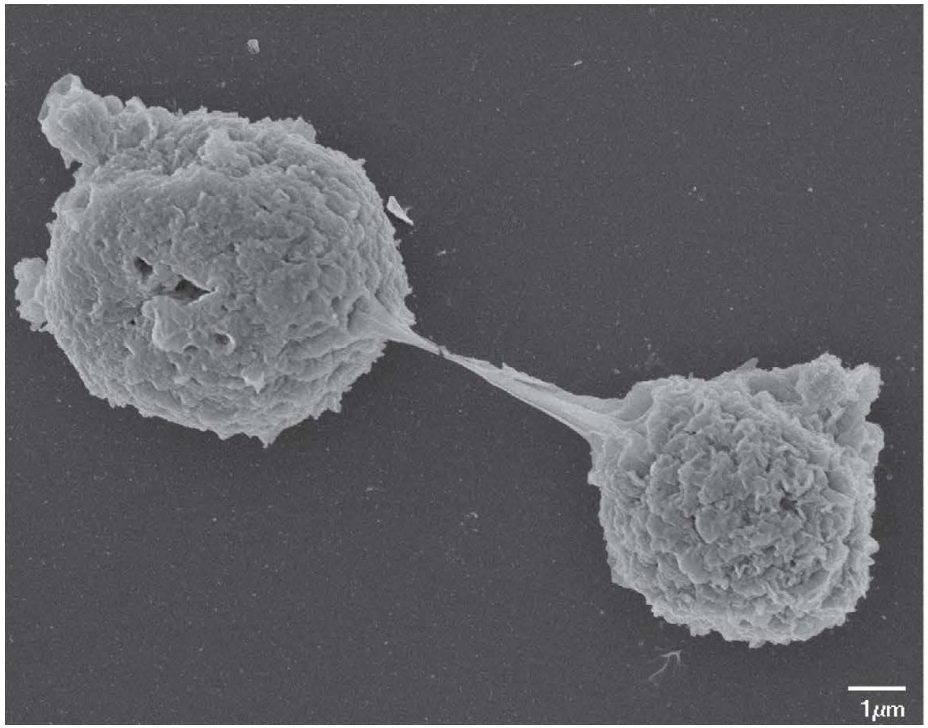
Vago и STAT-сигналинг

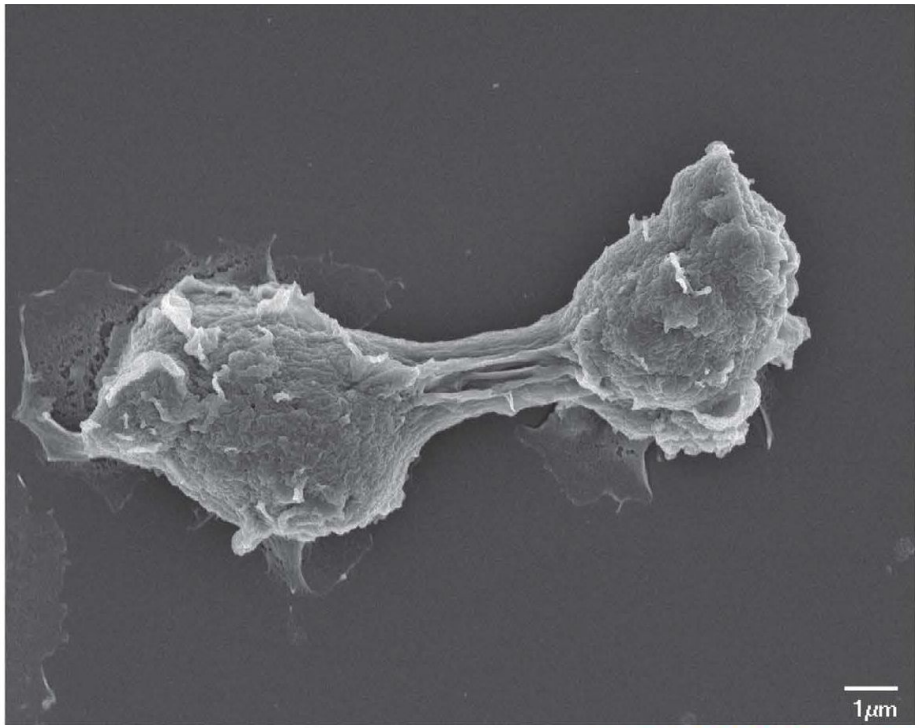


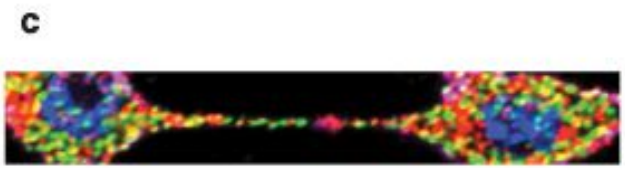
Системное действие РНК-интерференции у мух



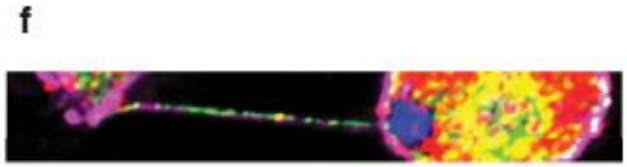
Клетки дрозофилы передают друг другу RNAi комплексы по нанотрубкам



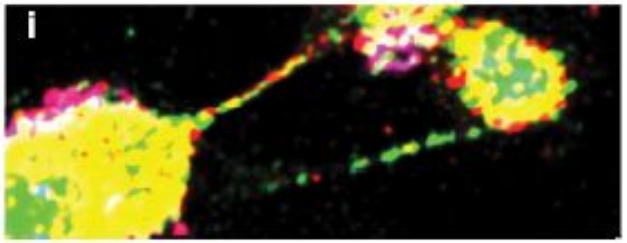




Rab7 / Ago2 / DAPI / Phalloidin

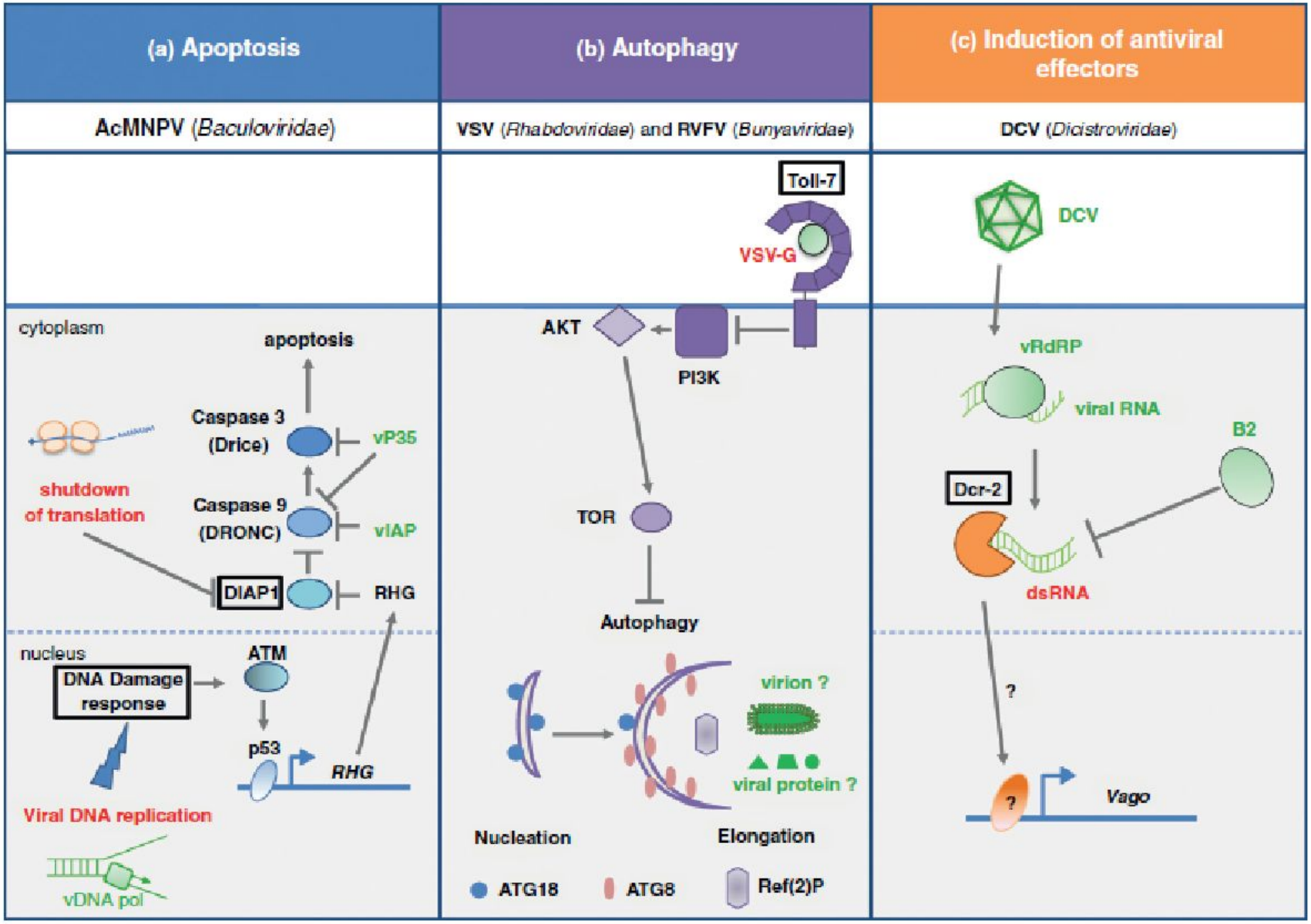


dsRNA / Ago2 / DAPI / Phalloidin

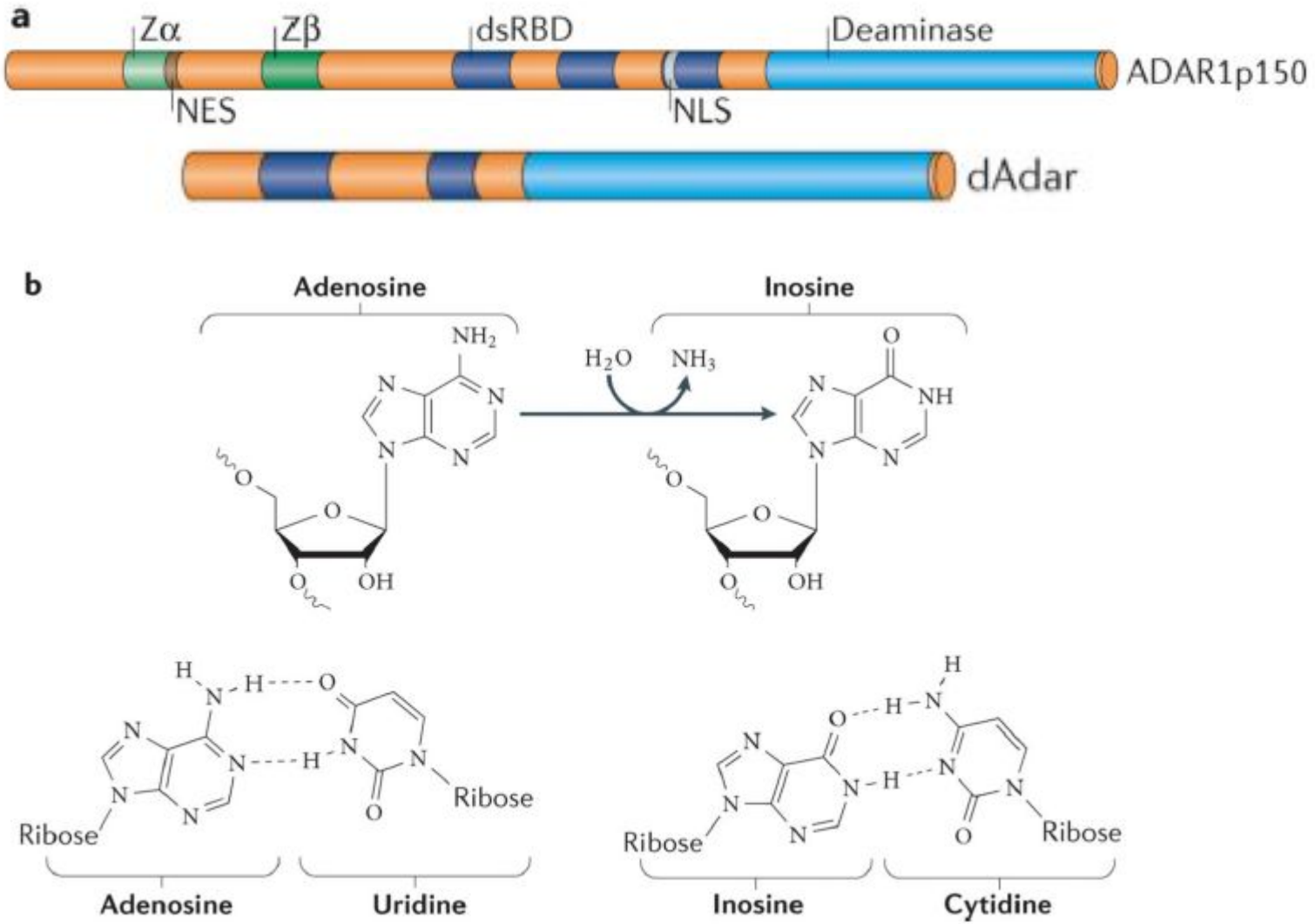


CG4572 / Ago2 / DAPI / Phalloidin

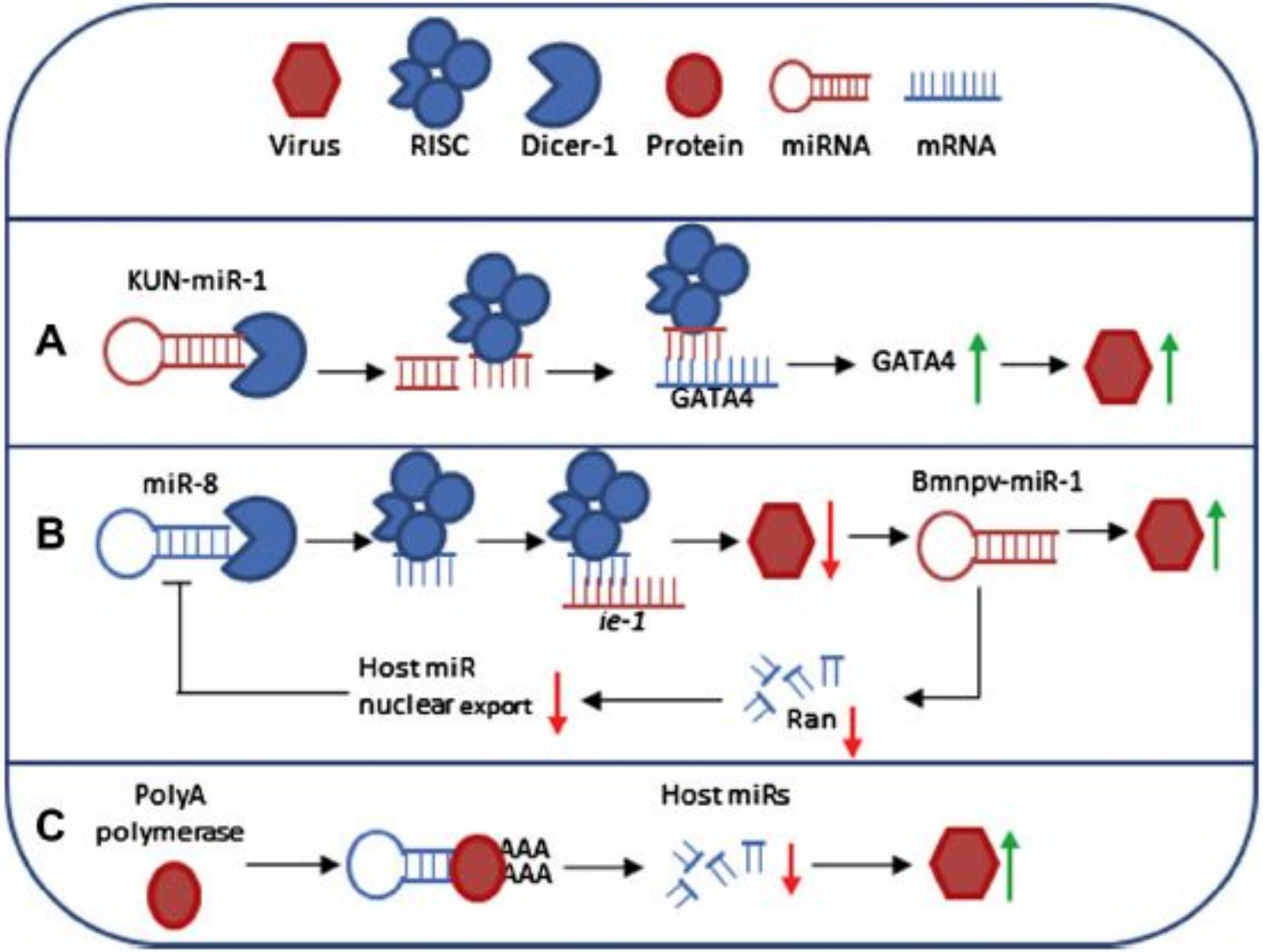
Варианты клеточного ответа на вирусную инфекцию у дрозофилы



Дезаминирование в молекуле РНК аденозина в инозин тоже может служить защитой от вирусов



К сожалению, РНК-интерференция – это игра, в которую может сыграть и сам вирус



Примеры молекул, с помощью которых вирусы манипулируют иммунитетом насекомых

Virus/bacteria-miRNA interactions in arthropods.

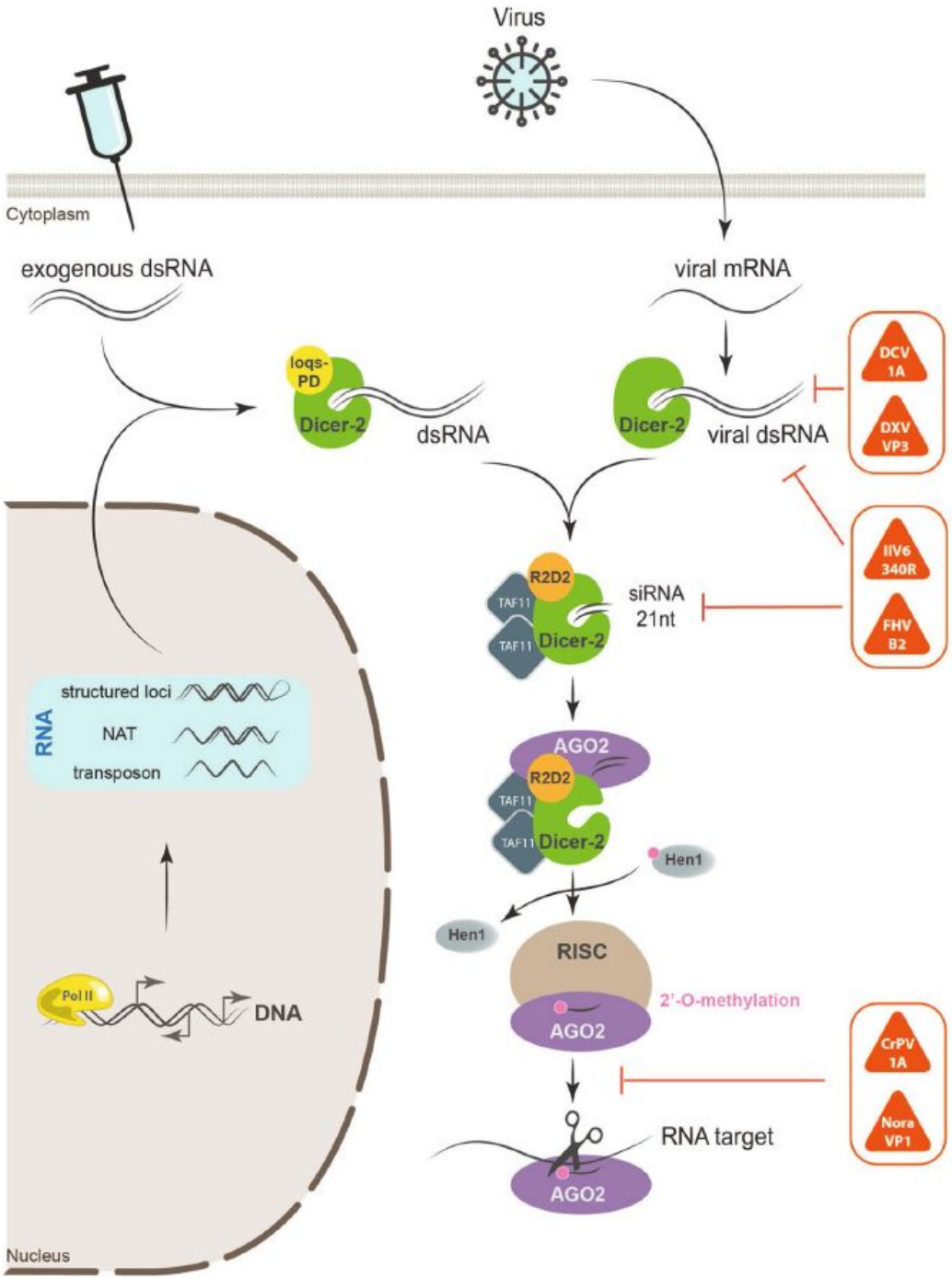
Virus/bacteria	Host insect	Insect factor	Virus factor	Mechanism/finding
Poxvirus (Vaccinia virus)	*Drosophila* DL1 cells	miR-34, miR-11, miR-184	Poly-A-Polymerase	Poly-A-polymerase poly adenylates miRs to lead to degradation
	Drosophila fat body	miR-8		miR-8 decreases drosomycin and diptericin AMPs in fat body, independent of PI3 K
Wolbachia	*Aedes aegypti*	Aae-miR-2940	Unknown	Wolbachia by an unknown mechanism induces miR-2940 which silences metalloprotease and increases bacterial density in the host
West Nile virus	Mosquito C6/36 cells	GATA4	KUN-miR-1	KUN-miR-1 leads to increased viral titer and accumulation of mosquito cell GATA4
Ascovirus	HzFB cells	Hz-miR24	RNApol	Host miR decreases expression of DNA dependent RNA polymerase in late infections
Bombyx mori baculovirus	*B. mori*	Exportin-5 miRNA export	Bmnpv-miR-1	Viral miRNA leads to RNAi repression of host Ran cofactor of exportin 5
Nudivirus (*HzNV-1*)	*Spodoptera frugiperda* IPLB-Sf-21		*Pag1 miRNA*	*Pag1* miRNA degrades viral early gene *hhi1* in latent infections

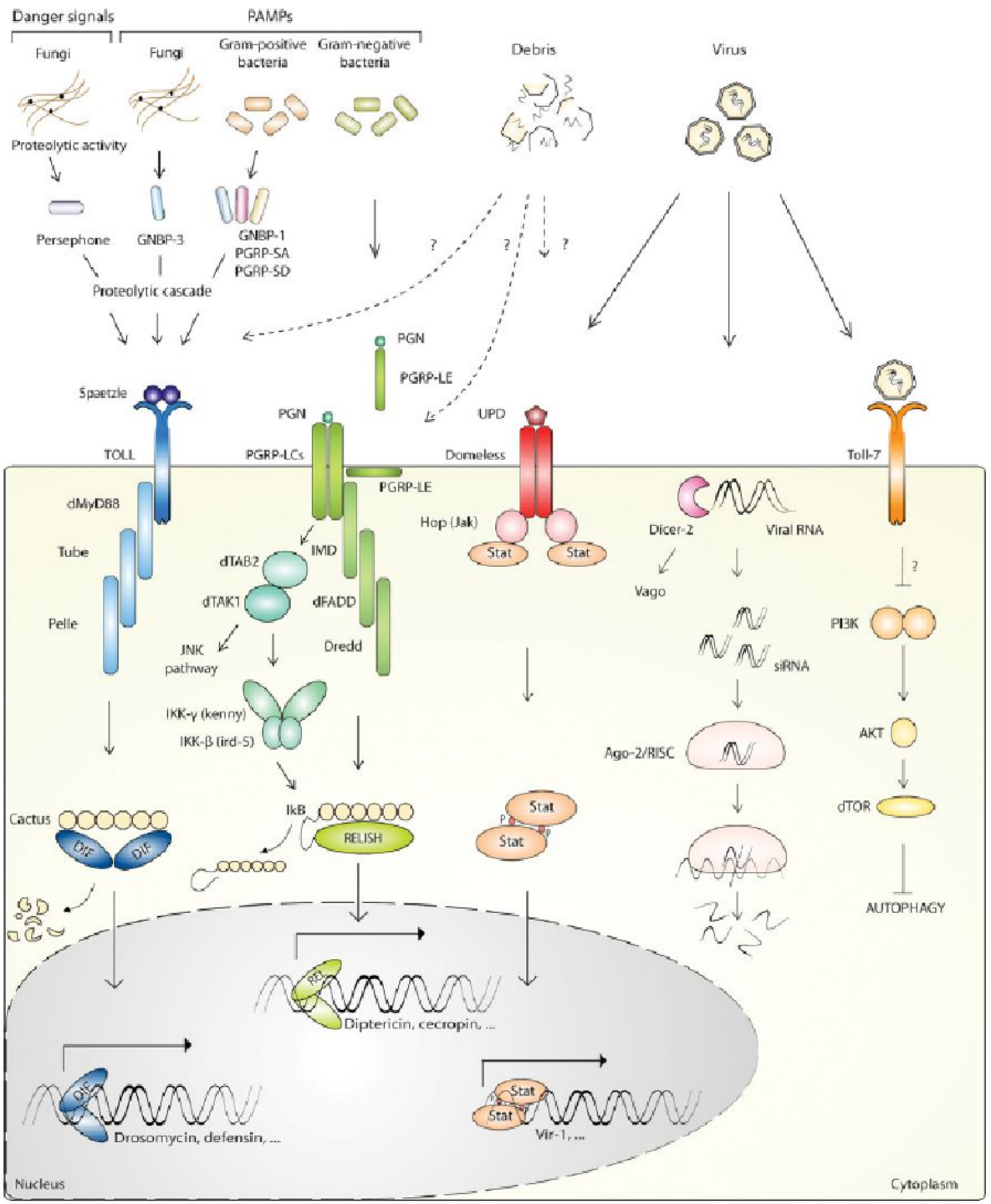
Arthropod virus-encoded protein or RNA suppressors of RNAi.

Virus	Antiviral suppressor	Mode of action
Flock house virus	B2 protein	Binds dsRNA and siRNAs
Drosophila C virus	DCV-1A protein	Inhibits Dicer-2 processing of dsRNA
Cricket paralysis virus	CrPV-1A protein	Interacts with Argonaute-2 to prevent RNA cleavage
Wuhan nodavirus	B2 protein	Prevents interaction of Dicer-2 with siRNA
West Nile virus	3′ UTR derived subgenomic flavivirus RNA (sfRNA)	Inhibits Dicer-2 processing of dsRNA (*in vitro*)

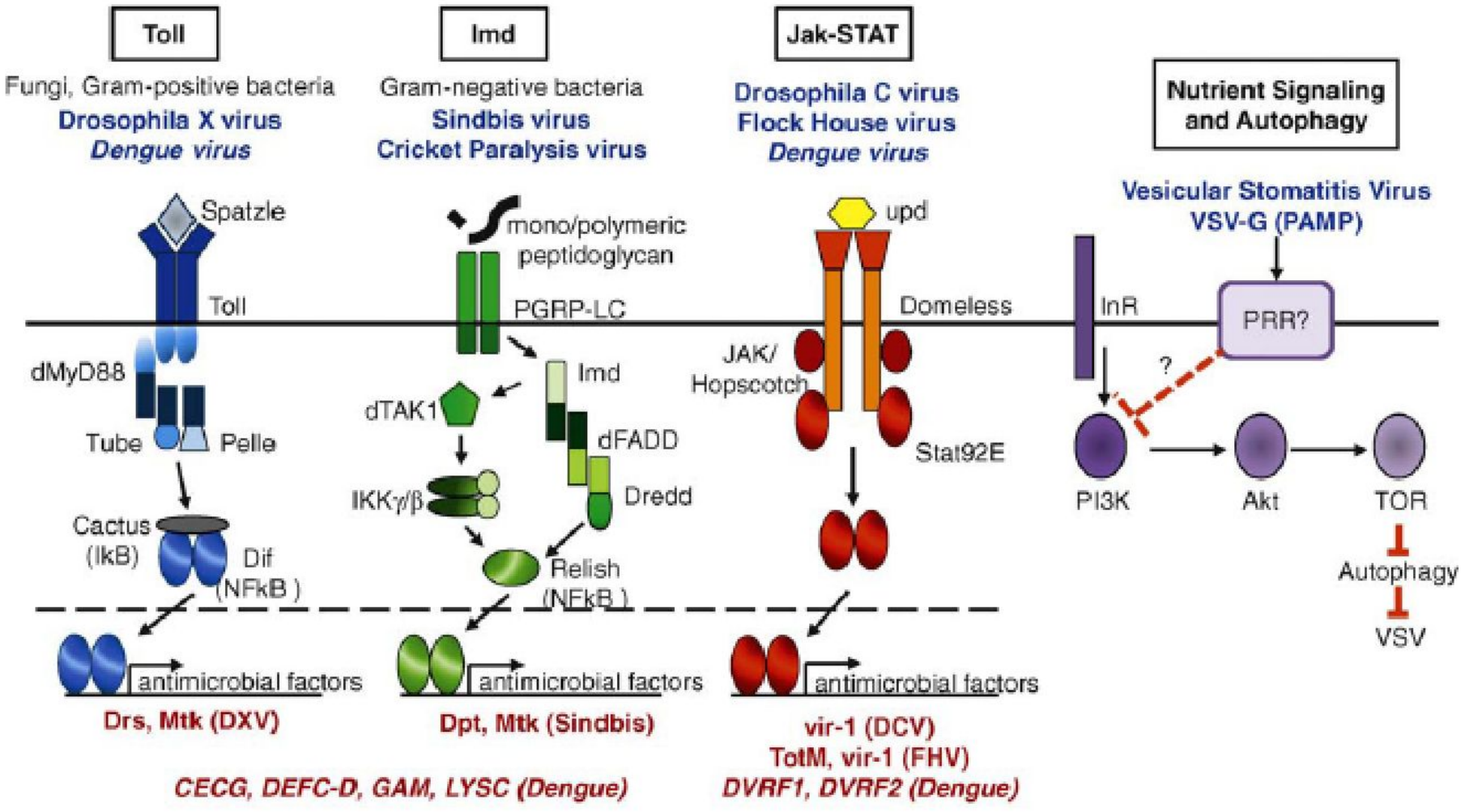
Viral RNAi suppressors in insect viruses and arboviruses

Family	Virus (abbr.)	Host/Vector[a,b]	Experimental insect host[c]	Suppressor	Mechanism
Ascoviridae	Heliothis virescens ascovirus-3e	*Heliothis virescens*	*Spodoptera frugiperda*; *Heliothis virescens*	Orf 27 (RNase III)	Degradation of dsRNA
Birnaviridae	Drosophila X virus	*D. melanogaster*	*D. melanogaster*	VP3	Long dsRNA binding prevents Dicer-2 cleavage; siRNA binding
	Culex Y virus	*Culex pipiens*	*Culex tarsalis*	VP3	Long dsRNA binding prevents Dicer-2 cleavage; siRNA binding
Dicistroviridae	Drosophila C virus (DCV)	*D. melanogaster*	*D. melanogaster*	1A	Long dsRNA binding prevents Dicer-2 cleavage; interferes with RISC assembly
	Cricket paralysis virus	*Teleogryllus* sp.	*D. melanogaster*	1A	Inhibition of AGO2 endonuclease activity
Flaviviridae	Dengue virus	*Ae. aegypti*; *Ae. albopictus*	*Spodoptera frugiperda*	NS4B	Inhibition of (human) Dicer activity
	West Nile virus	*Culex* spp.	*Ae. Albopictus*; *D. melanogaster*	sfRNA	Inhibition of (human) Dicer activity
	Dengue virus	*Ae. aegypti*; *Ae. albopictus*	*Ae. albopictus*	sfRNA	–
Nodaviridae	Flock House virus (FHV)	*Costelytra zealandica*	*D. melanogaster*; *Spodoptera frugiperda*; *Ae. aegypti*; *Ae. albopictus*	B2	Long dsRNA binding prevents Dicer-2 cleavage; siRNA binding prevents RISC incorporation; Dicer-2 binding
	Nodamuravirus (NoV)	*Culex tritaeniorhynchus*	*D. melanogaster*; *An. gambiae*; *Ae. albopictus*	B2	Binding of long dsRNA and siRNA; inhibition of (human) Dicer activity
	Wuhan Nodavirus (WhNV)	*Pieris rapae*	*Pieris rapae*; *D. melanogaster*	B2	Long dsRNA binding prevents Dicer-2 cleavage; siRNA binding prevents RISC incorporation; Dicer-2 binding


Virus
Cytoplasm
exogenous dsRNA
viral mRNA
loqs-PD
Dicer-2
dsRNA
Dicer-2
viral dsRNA
DCV 1A
DXV VP3
IIV6 340R
FHV B2
R2D2
TAF11
TAF11
Dicer-2
siRNA 21nt
AGO2
R2D2
TAF11
TAF11
Dicer-2
Hen1
Hen1
RISC
2'-O-methylation
AGO2
CrPV 1A
Nora VP1
RNA target
AGO2
RNA
structured loci
NAT
transposon
Pol II
DNA
Nucleus


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
TOLL
dMyD88
Tube
Pelle
PGN
PGRP-LCs
PGRP-LE
IMD
dTAB2
dTAK1
dFADD
Dredd
JNK pathway
IKK-γ (kenny)
IKK-β (ird-5)
Cactus
DIF
DIF
IκB
RELISH
UPD
Domeless
Hop (Jak)
Stat
Stat
Dicer-2
Viral RNA
Vago
siRNA
Ago-2/RISC
Toll-7
PI3K
AKT
dTOR
AUTOPHAGY
Stat
Stat
Diptericin, cecropin, ...
DIF
Drosomycin, defensin, ...
Stat
Stat
Vir-1, ...
Nucleus
Cytoplasm

Вирусы дрозофилы и активируемые ими пути сигналинга

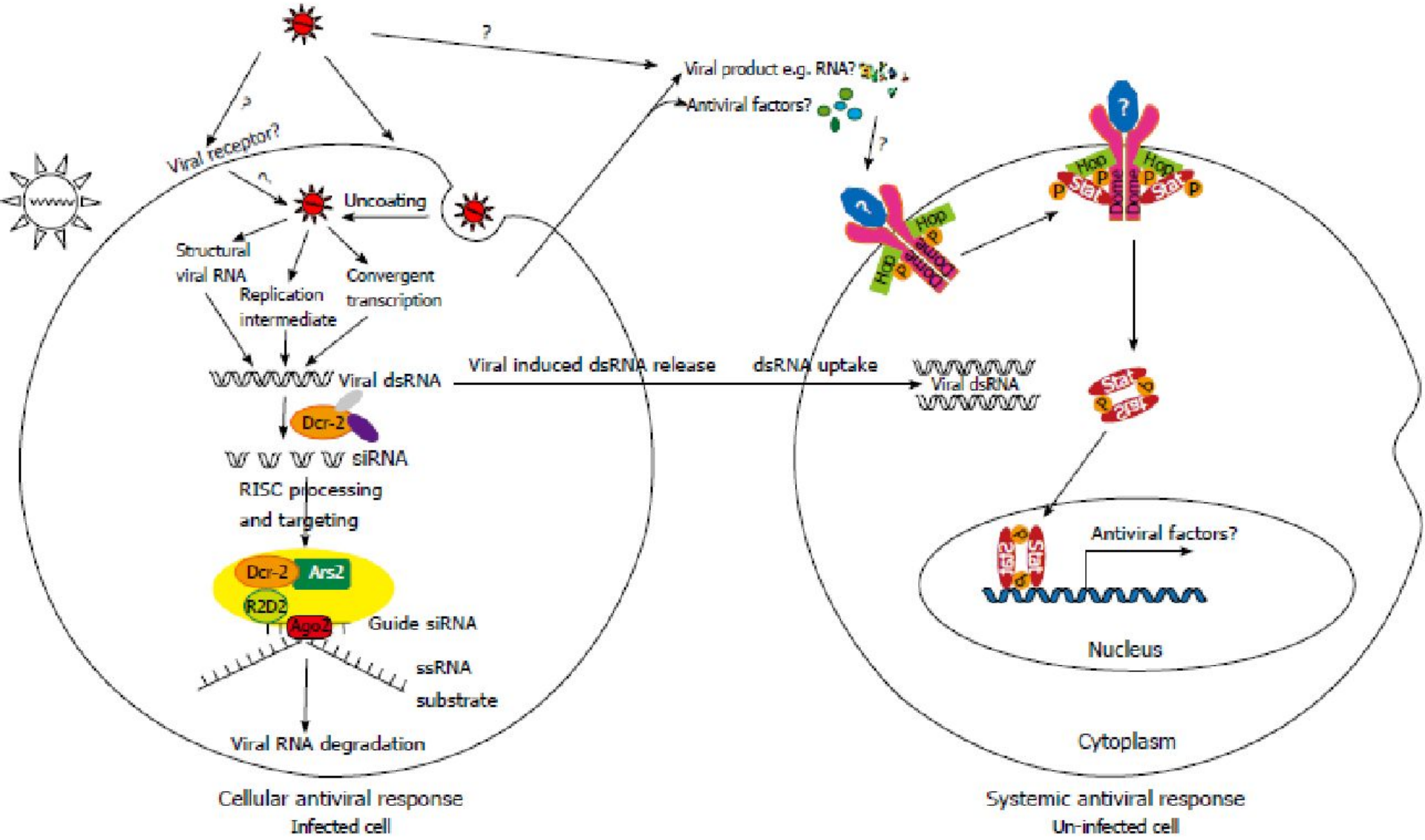


Против вирусов также могут быть использованы пути Toll и Imd

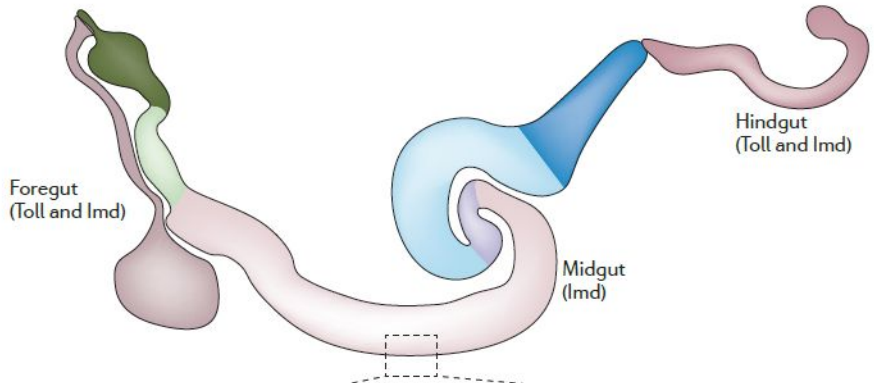
Inducible immune pathways implicated in antiviral defense in *Drosophila* and mosquito.

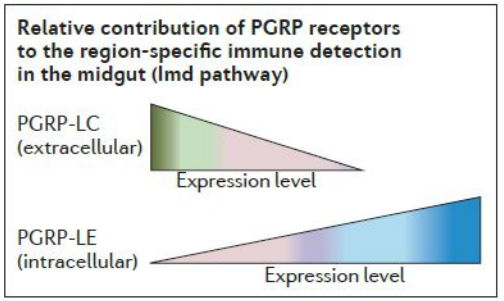
Pathway	Organism	Virus	Inducible immune response			
			Genetic evidence[a]	Transcriptional response		
				Pathway components[b]	AMP expression[c]	Other humoral factors[d]
Toll	*Drosophila melanogaster*	DXV	+	n.t.	Up	n.a.
	Aedes aegypti	DENV	+	Up	Up	n.a.
		DENV	n.t.	Down	–	n.a.
		SINV	n.t.	Up	n.t.	n.a.
		YFV	n.t.	Down	–	n.a.
	Aedes albopictus	SFV[e]	n.t.	n.t.	Down[f]	n.a.
Imd	*Drosophila melanogaster*	CrPV	+	n.t.	–	n.a.
		DmelSV[g]	n.t	Up[g]	Up[g]	n.a.
		SINV[h]	+	n.t.	Up	n.a.
	Aedes albopictus	SFV[e]	n.t.	n.t.	Down[f]	n.a.
	Anopheles gambiae	ONNV	-	Down	–	n.a.
Jak-Stat	*Drosophila melanogaster*	DCV	+	–	–	Up
		FHV	n.t.	n.t.	–	Up
	Aedes aegypti	DENV	+	Up	Up	Up
		DENV	n.t.	–	Down[i]	–
		WNV	n.t.	–	Down[i]	–
		YFV	n.t.	–	Down[i]	–
	Aedes albopictus	SFV[e]	n.t.	n.t.	Down[f]	n.t.

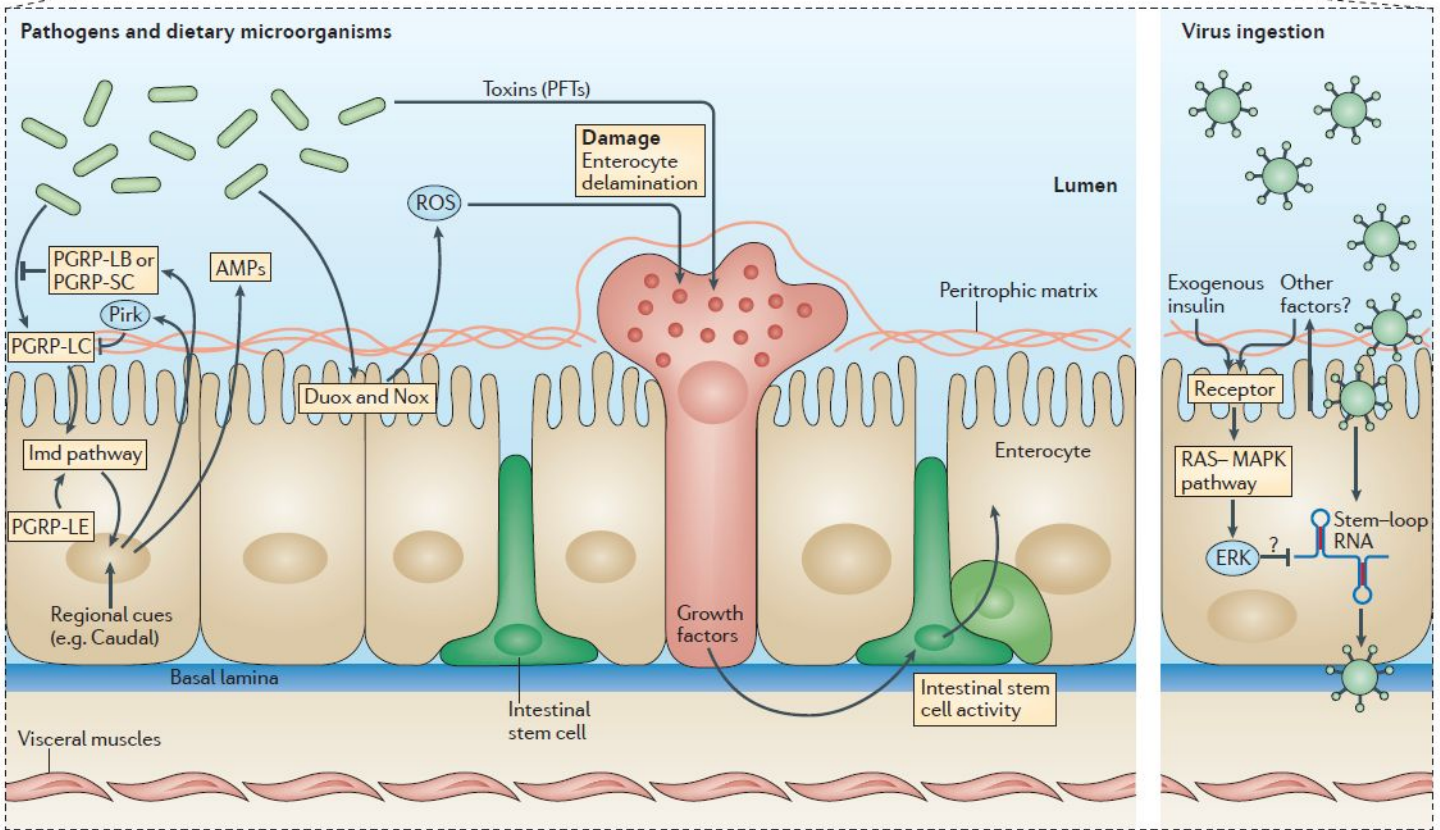
Механизмы противовирусной защиты – обобщение



Иммунитет кишечника мухи







Структура гена белка Dscam дрозофилы

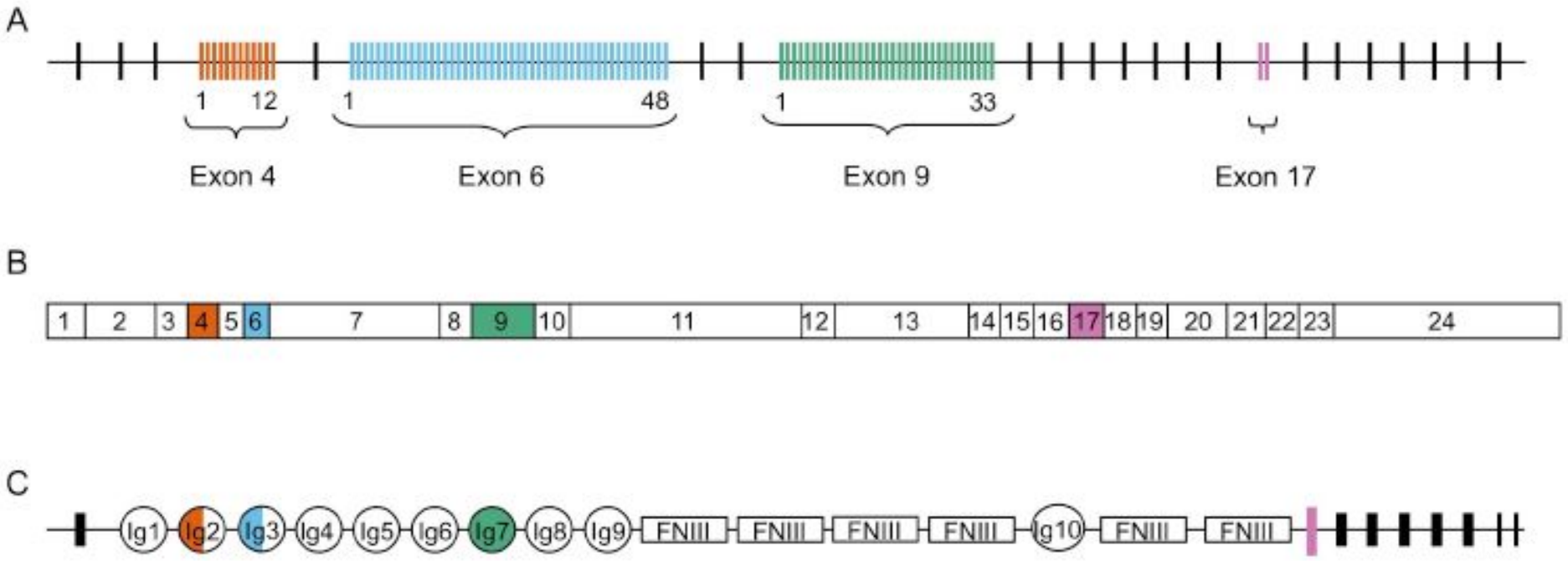
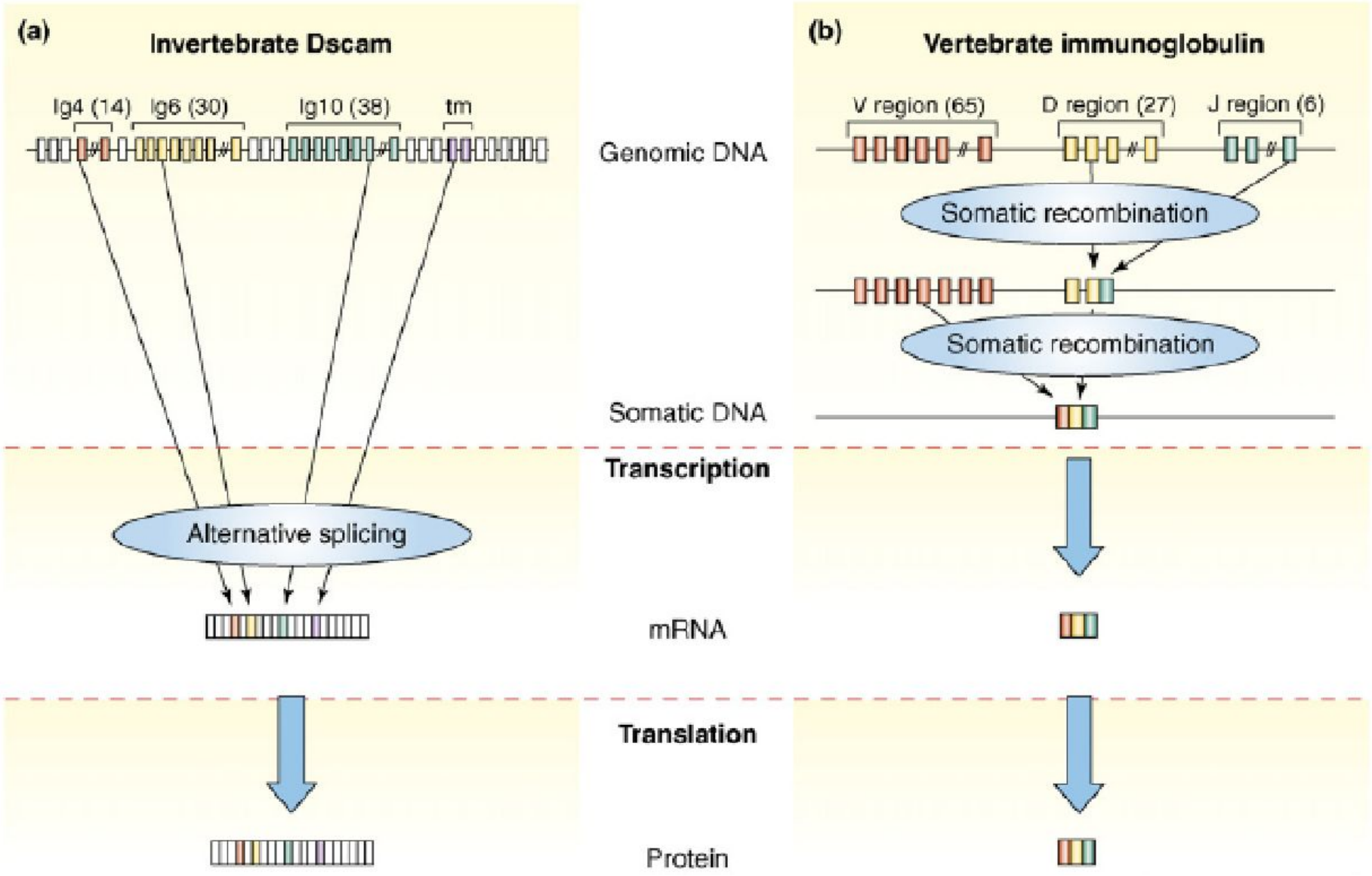
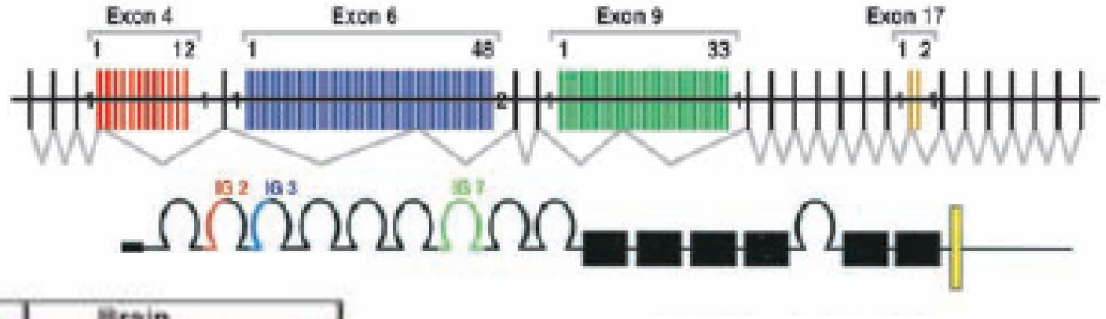


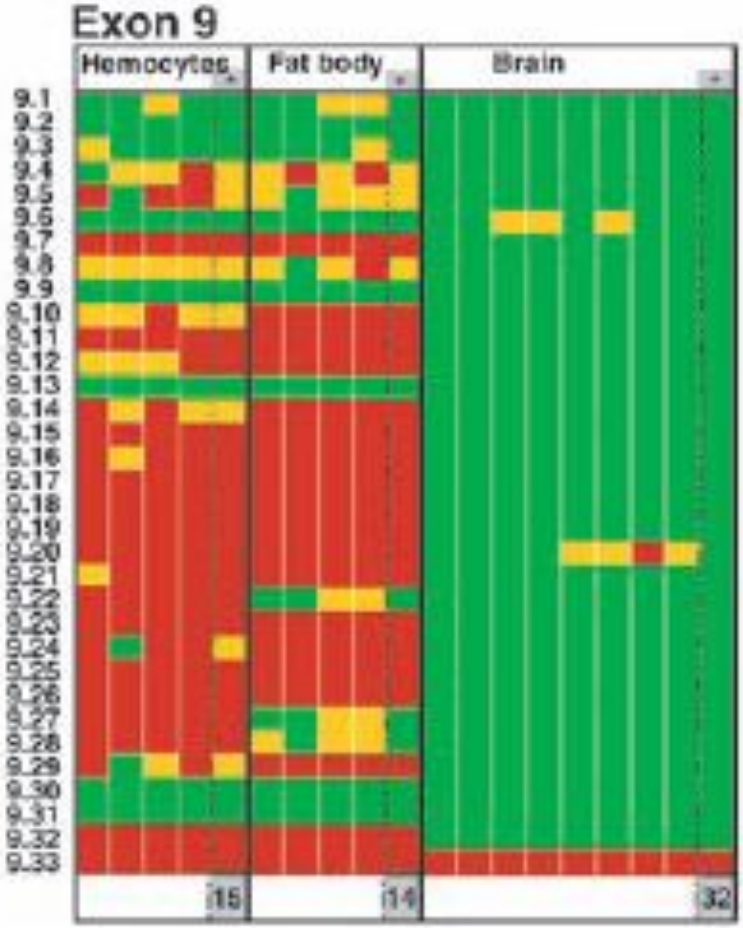
Figure 1 (A) *Dscam-hv* genomic DNA for *Drosophila melanogaster*. The gene consists of 20 constant exons (shown as black lines), mutually exclusive alternative splicing occurs for exons 4 (red lines), 6 (blue lines), 9 (green lines) and 17 (purple lines); one of 12 exon 4 alternatives, one of 48 exon 6 alternatives, one of 33 exon 9 alternatives and one of two exon 17 alternatives are present in each mRNA. This enables the vast number of $12 \times 48 \times 33 \times 2 = 38{,}016$ potential splice variants. **(B) *Dscam-hv* mRNA.** Constant exons are shown as white boxes. Exons that undergo mutually exclusive alternative splicing follow the same colour scheme as for the genomic structure. Endodomain exons 19 and 23 can be contained or lacking [8], which increases the number of potential isoforms to $4 \times 38{,}016 = 152{,}064$. **(C) Dscam-hv protein structure for *D. melanogaster*.** The alternatively spliced exons encode the N-terminal half of Ig2 (exon 4 in *Drosophila*); the N-terminal half of Ig3 (exon 6 in *Drosophila*), all of Ig7 (exon 9 in *Drosophila*), and the transmembrane domain (Exon 17 in *Drosophila* (figure after [6]).

Dscam и иммуноглобулины позвоночных



В иммунной системе мухи Dscam обладает дифференциальной экспрессией





Что мы можем интерпретировать в пользу роли Dscam в иммунитете

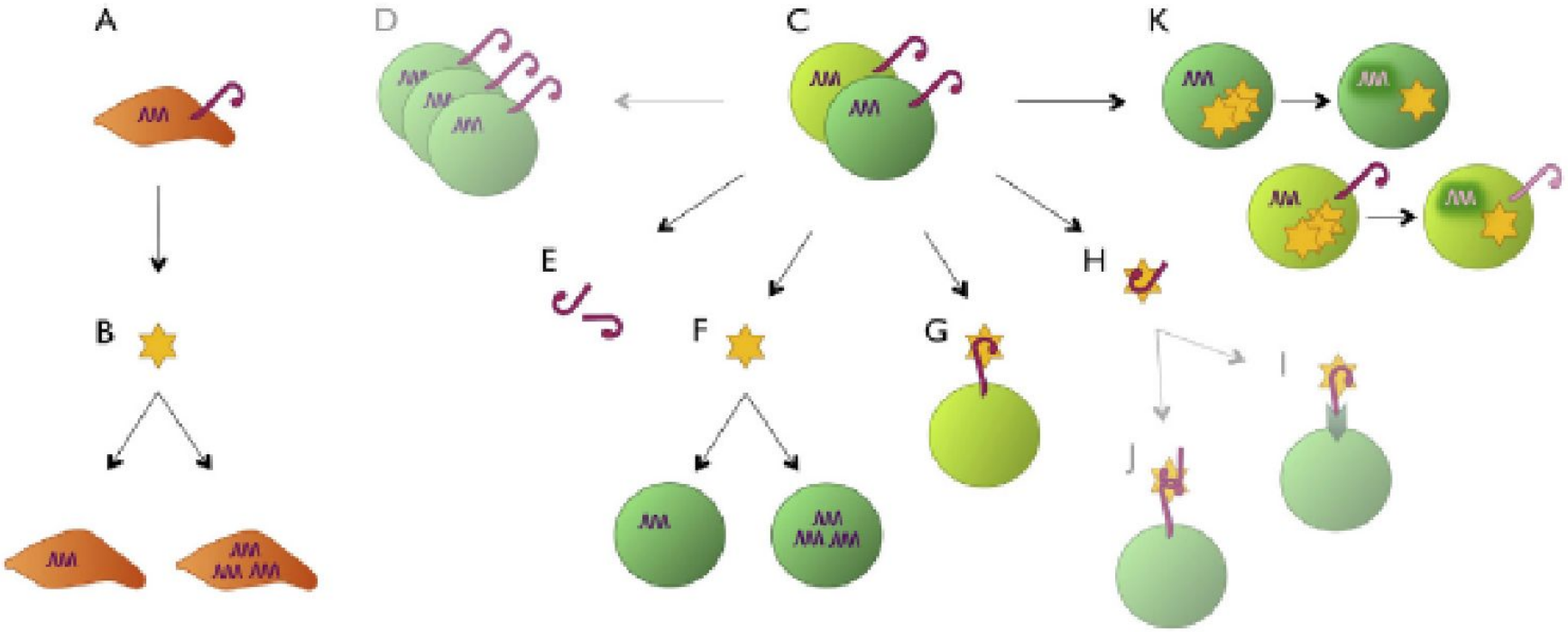
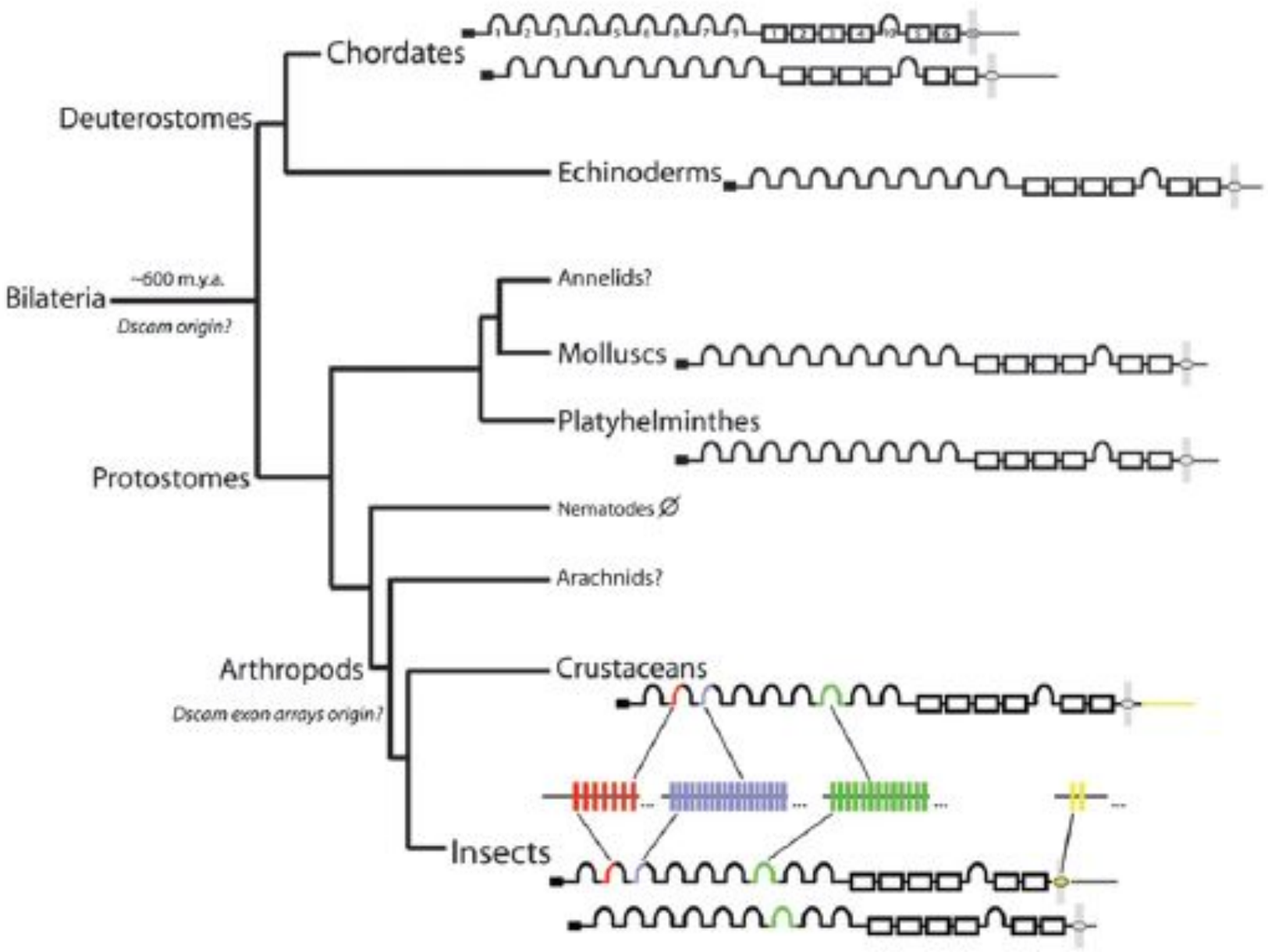
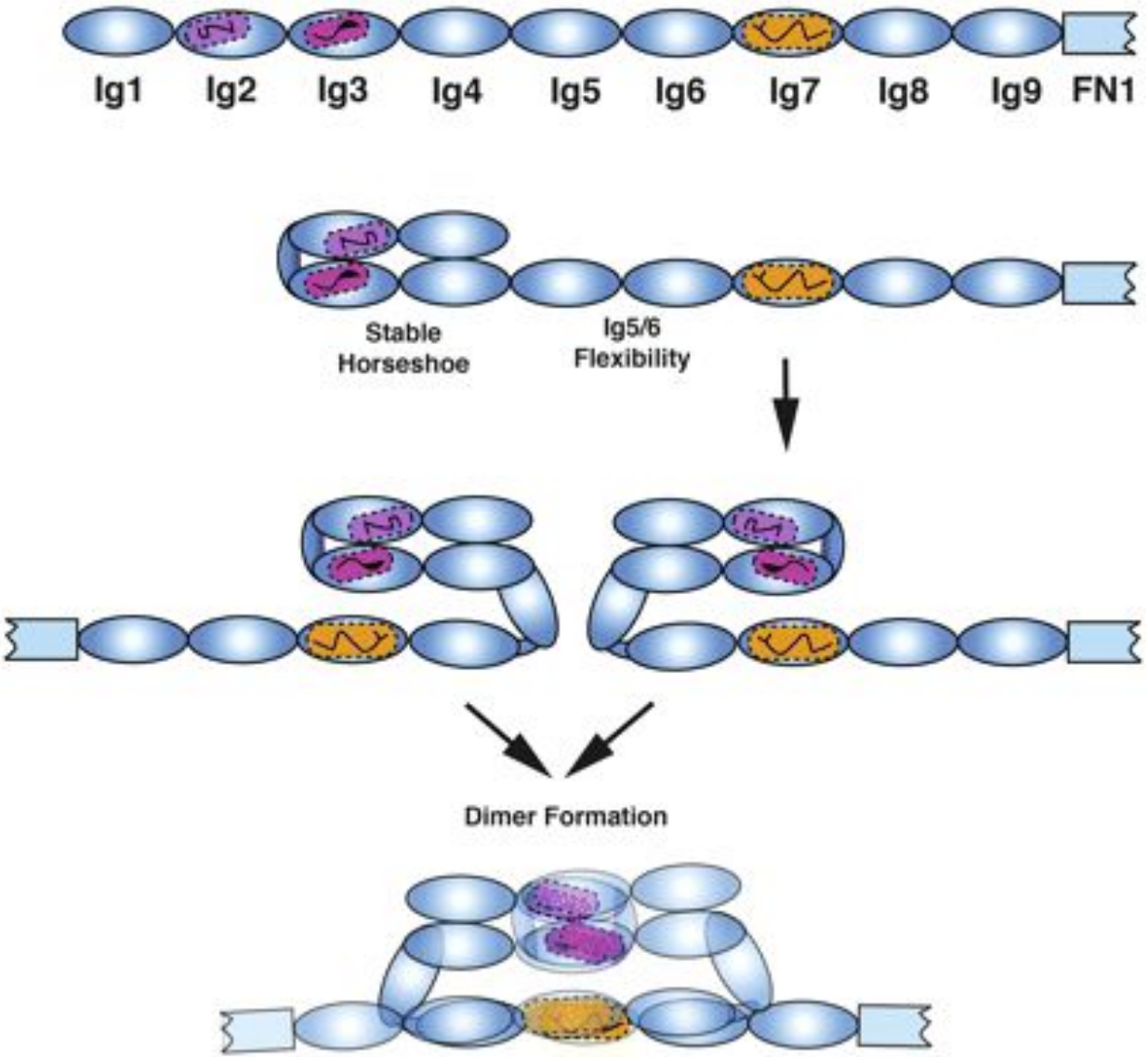


Fig. 2. Scheme of some experimentally supported (letters shown in black) and hypothesised (letters shown in grey) activities of *Dscam* in relation to pancrustacean immune tissues and responses to parasites. *Dscam* mRNA is shown as a squiggle inside the cells and the protein as a hook, with the first four Dscam Ig domains illustrated in a horseshoe shape (Meijers et al., 2007). For simplicity, we have omitted illustrating the expression of alternatively spliced variants and the regulation of splicing (e.g., Dong et al., 2012); details can be found in Table 1. (A) *Dscam* is expressed by the fat body (Watson et al., 2005). (B) Fat body *Dscam* mRNA expression has been found to be up- or non-regulated (see Table 1) after exposure to parasites (yellow star). (C) *Dscam* is also expressed by haemocytes (dark green; Neves et al., 2004; Watson et al., 2005) and insect cell lines (light green; Watson et al., 2005; Dong et al., 2006; Sun et al., 2013). It has been hypothesised that increased expression of certain *Dscam* isoforms may occur via clonal expansion of (D), haemocytes (Boehm, 2007). (E) A shorter Dscam protein form has been found in S2 cell line-conditioned medium and haemolymph serum (Watson et al., 2005). (F) Haemocyte *Dscam* mRNA expression has been found to be up- down- or non-regulated after exposure to parasites (see Table 1). (G) Sua5B cell line membrane-bound Dscam shows affinity to *E. coli* and *P. veronii* (Dong et al., 2006). (H) Recombinantly expressed Dscam protein can bind to parasites (Watson et al., 2005; Watthanasurorot et al., 2011; Hung et al., 2013), and Dscam expressed in the midgut colocalises with *Plasmodium falciparum* (Dong et al., 2012). It is unknown whether Dscam acts as an opsonin, but if so, it has been hypothesised that it could (I), present the pathogen to a so far unknown Dscam receptor (Stuart and Ezekowitz, 2008) or (J), that there could be a homotypic interaction with membrane-bound Dscam (Stuart and Ezekowitz, 2008). (K) *Dscam* reduced (via RNAi/mutation/using Dscam antibodies to block Dscam function) haemocytes and cells lines show reduced phagocytosis of bacteria (Watson et al., 2005; Dong et al., 2006). (Figure updated from Boehm, 2007 and Stuart and Ezekowitz, 2008).

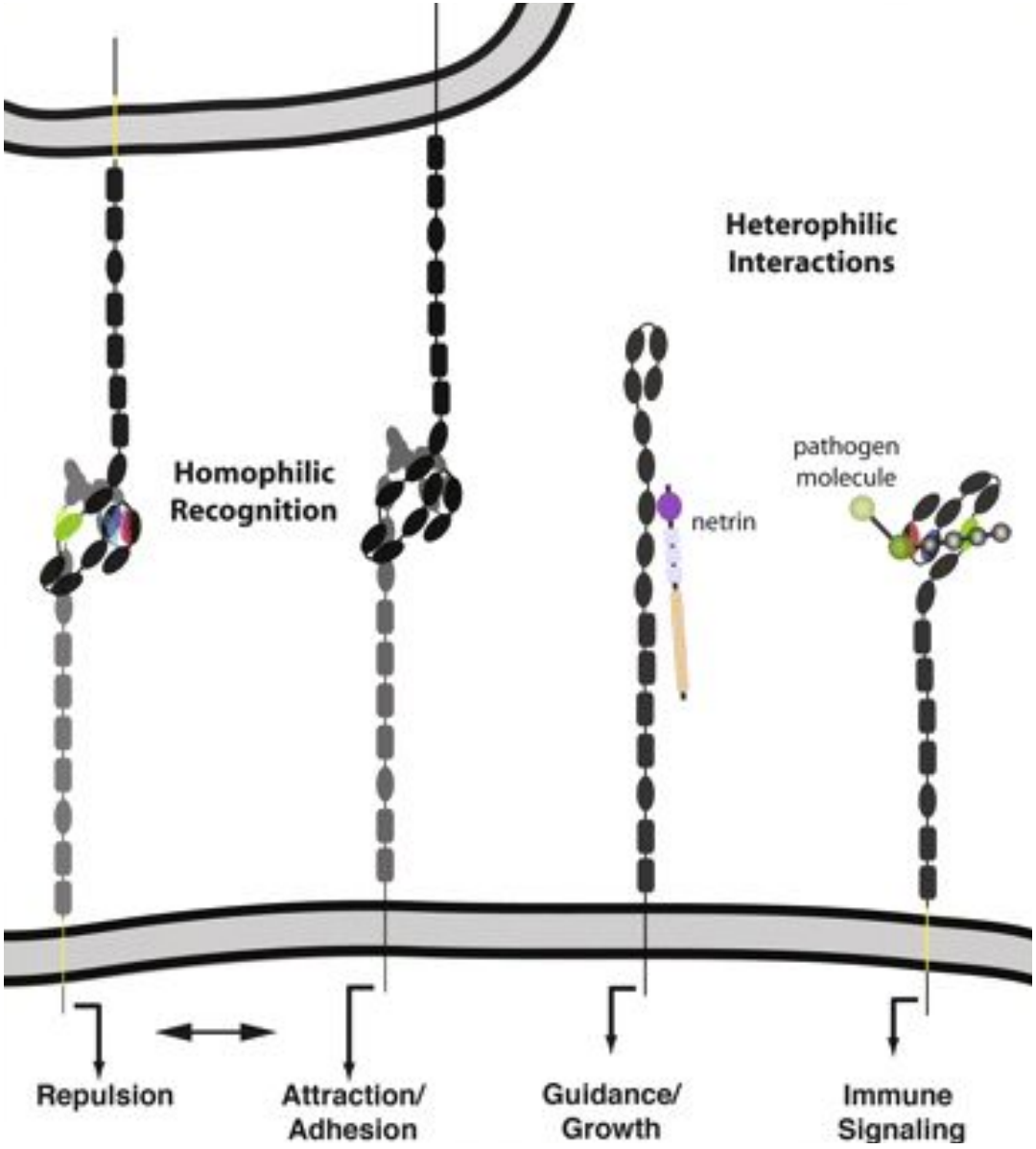
Только членистоногие имеют кассеты экзонов в гене DSCAM



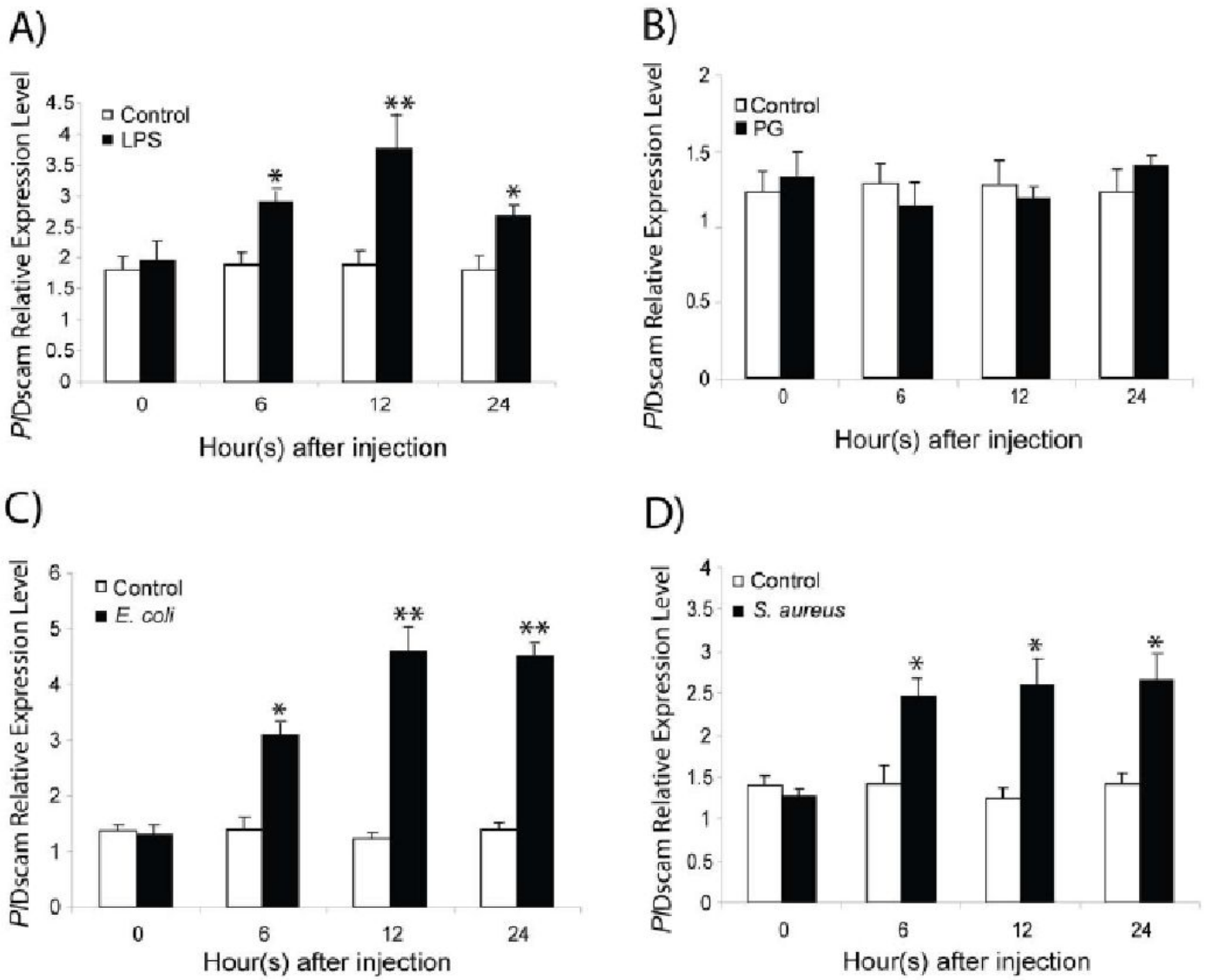
Гомофильные взаимодействия Dscam…



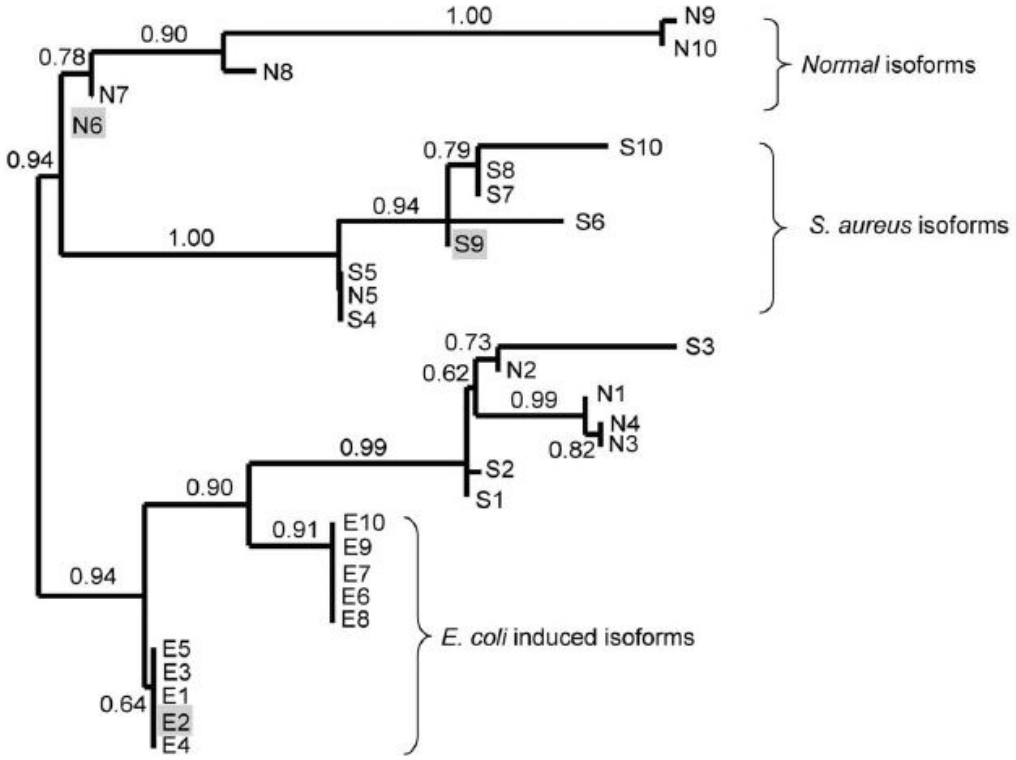
…и гетерофильные взаимодействия



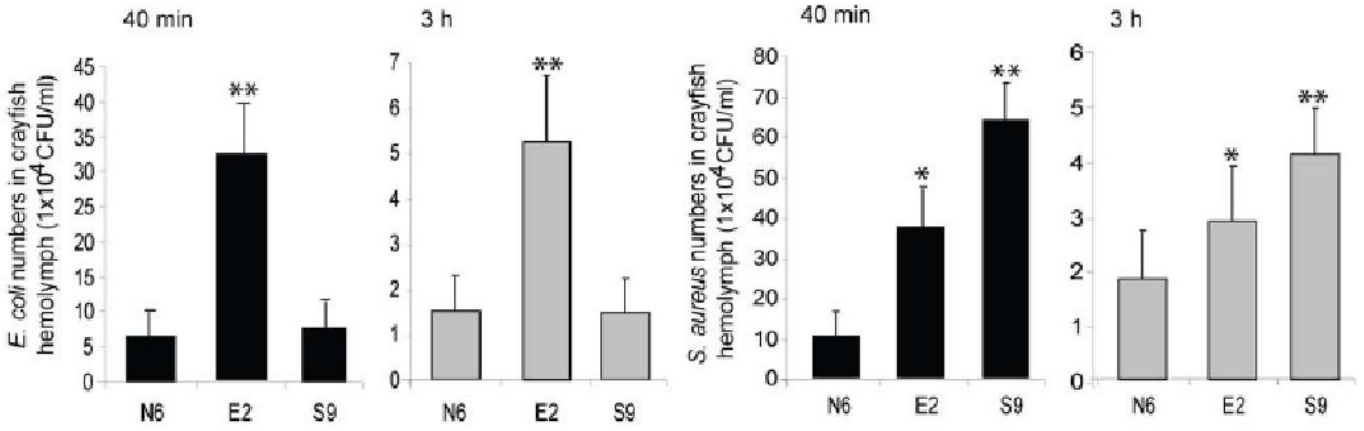
Для анофелеса и речного рака есть данные, указывающие на повышение экспрессии Dscam в присутствии патогенов



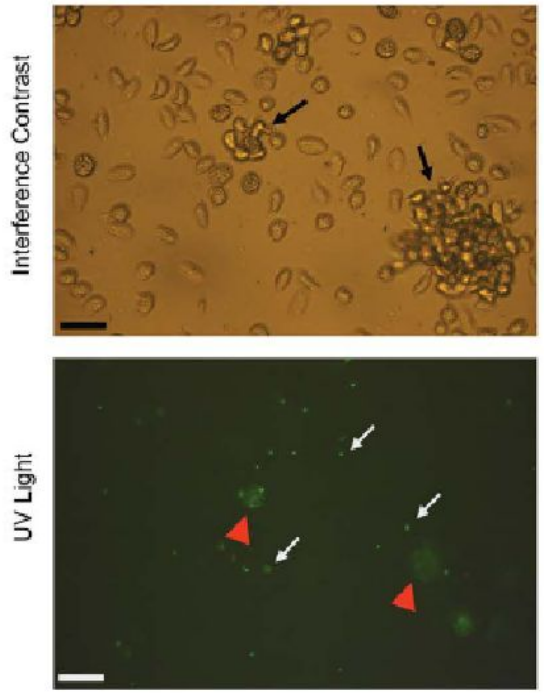
При этом после инъекции патогена в гематоцитах экспрессируются изоформы Dscam, отличающиеся от рутинных



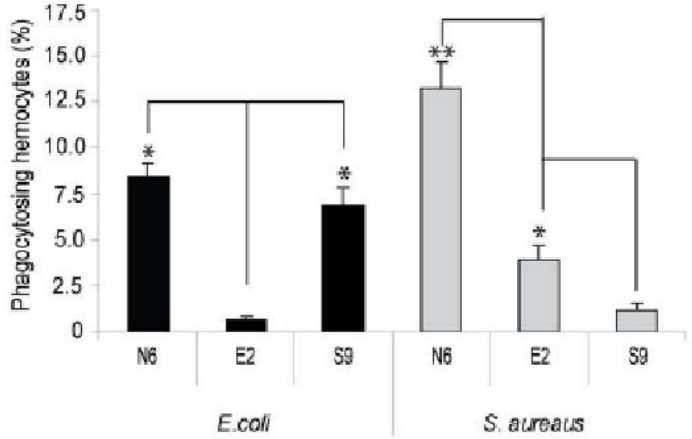
Обработанные рекомбинантными Dscam-белками бактерии подавляют фагоцитарную активность гематоцитов и дольше выживают в гемолимфе



E)

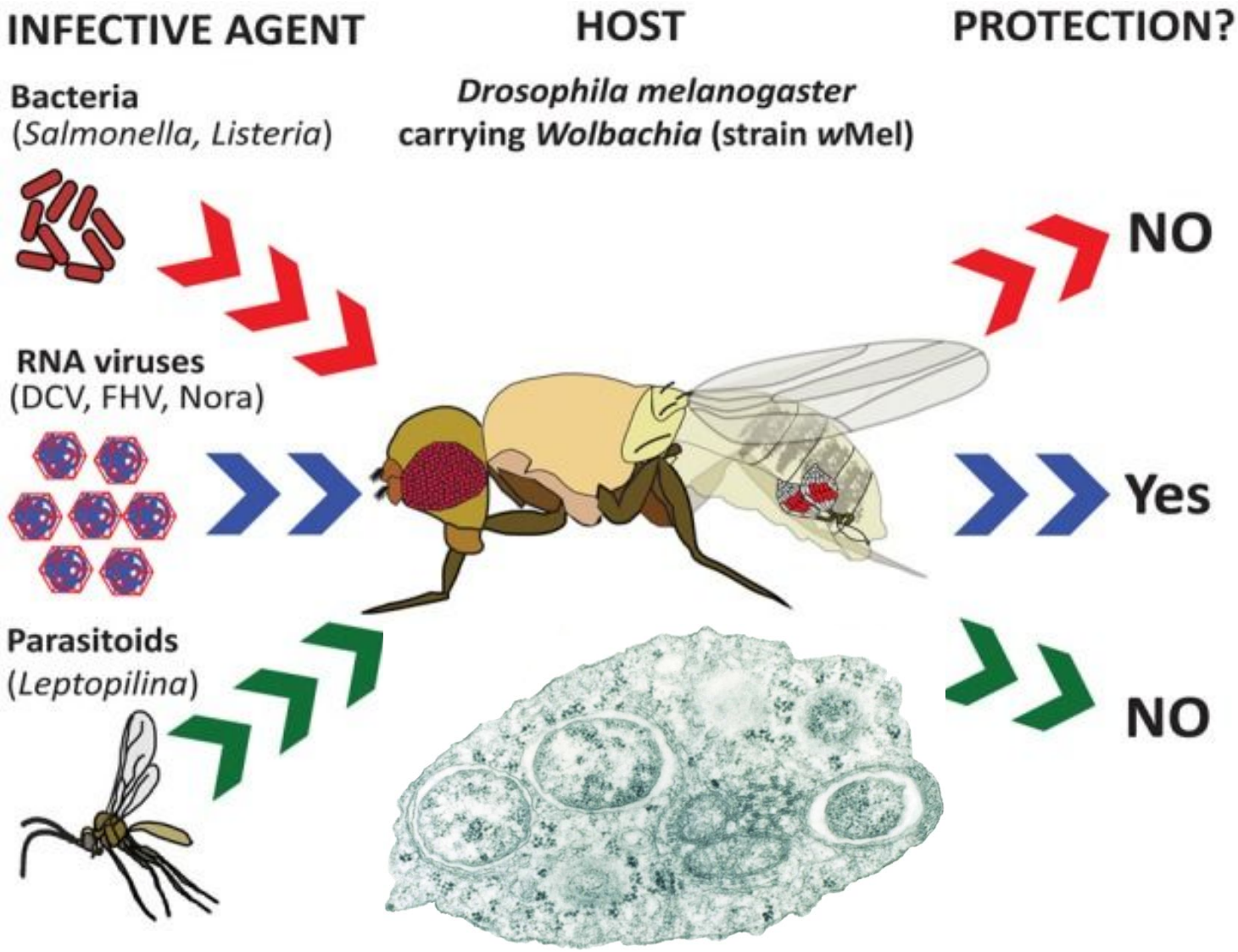


F)



Однако прямых доказательств того, что Dscam является патоген-распознающим рецептором нет.

Вольбахия защищает дрозофил от вирусов?



- Следующая лекция 19 марта – об иммунных системах других беспозвоночных