

Лекция 5 «Иммунитет дрозозифилы»



Шилов Е.С.
12 марта 2018

Иммуноциты дрозодилы

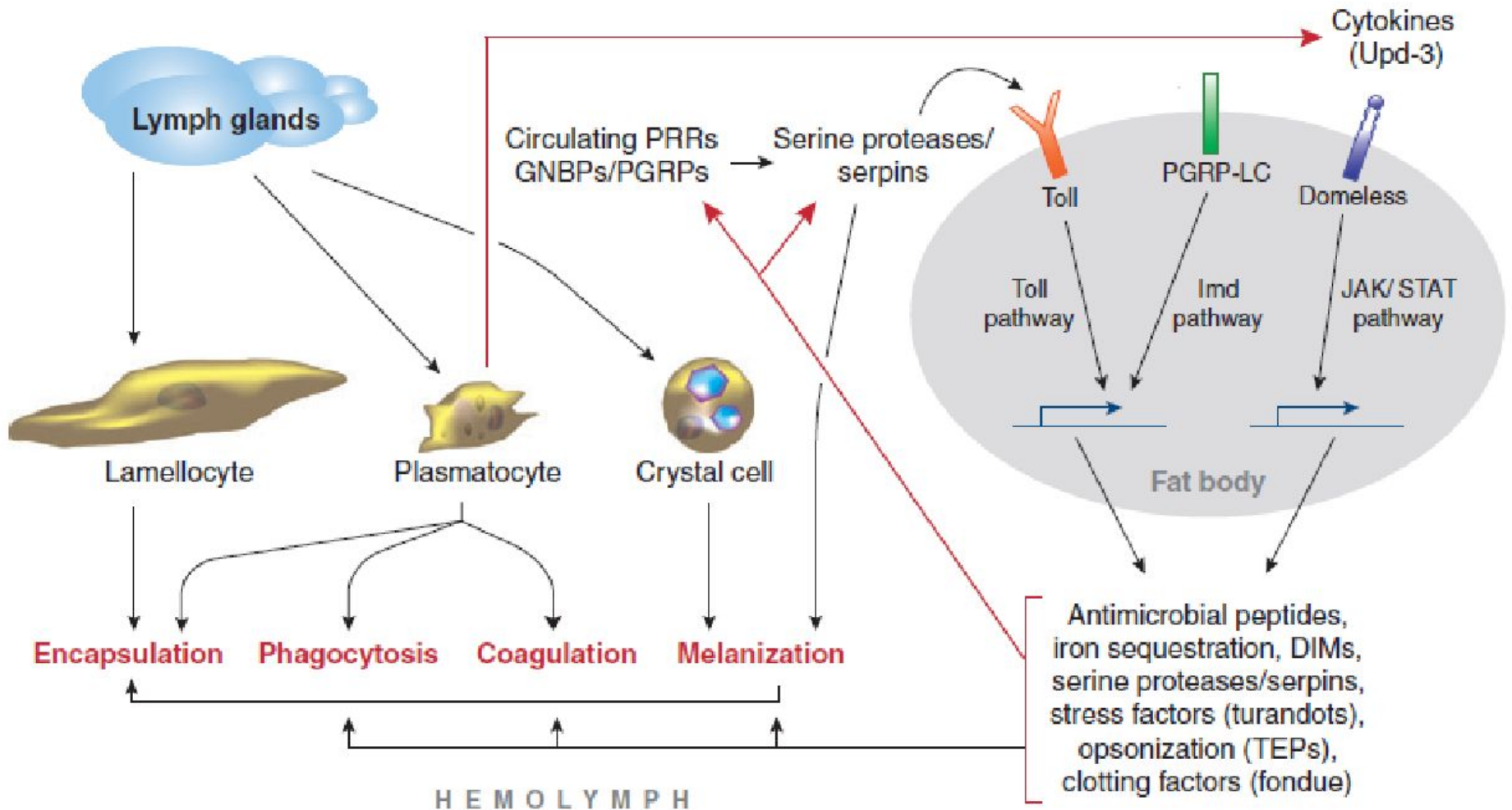
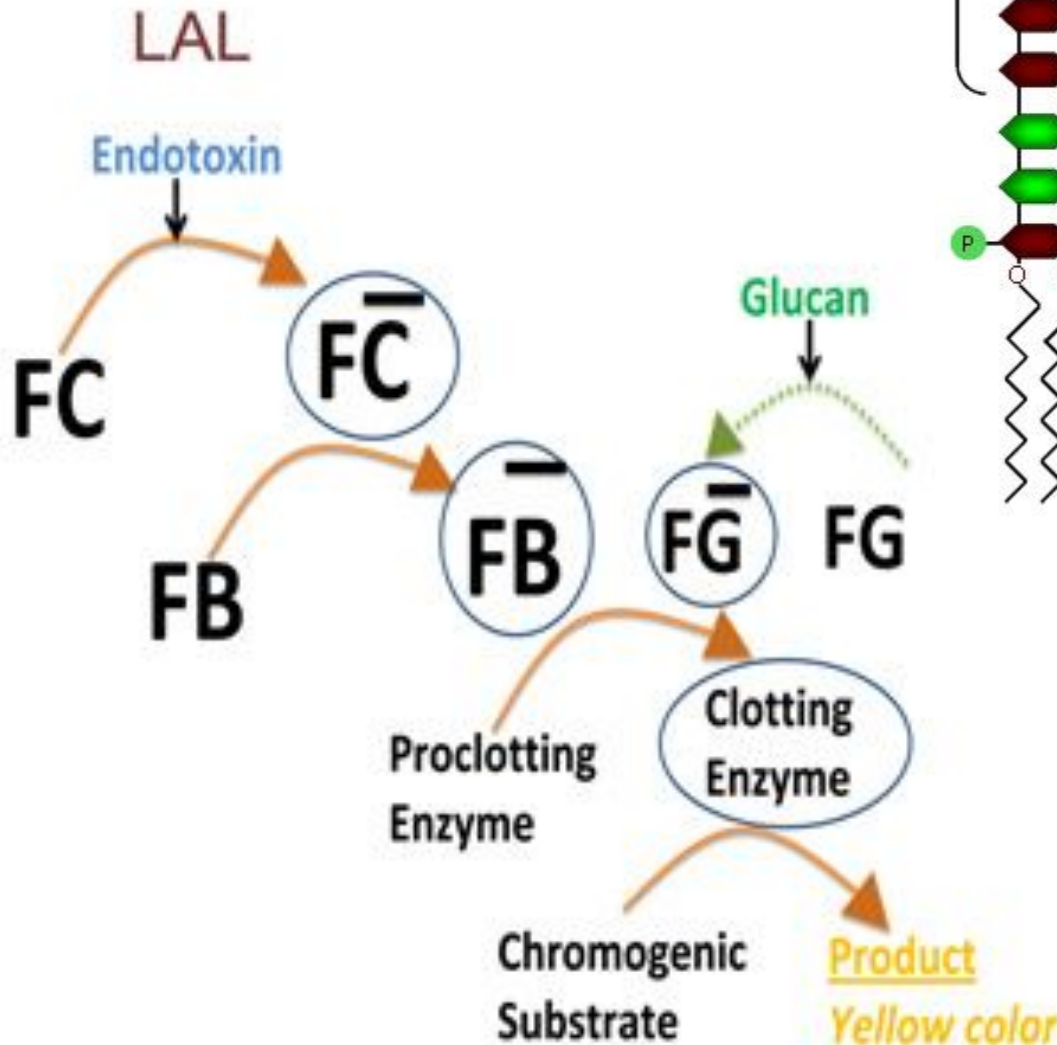


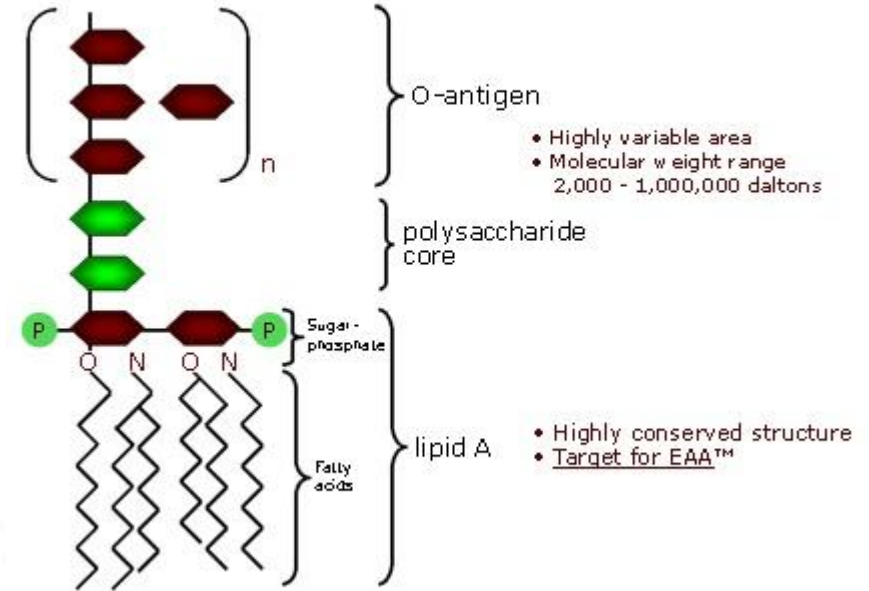
Figure 1

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

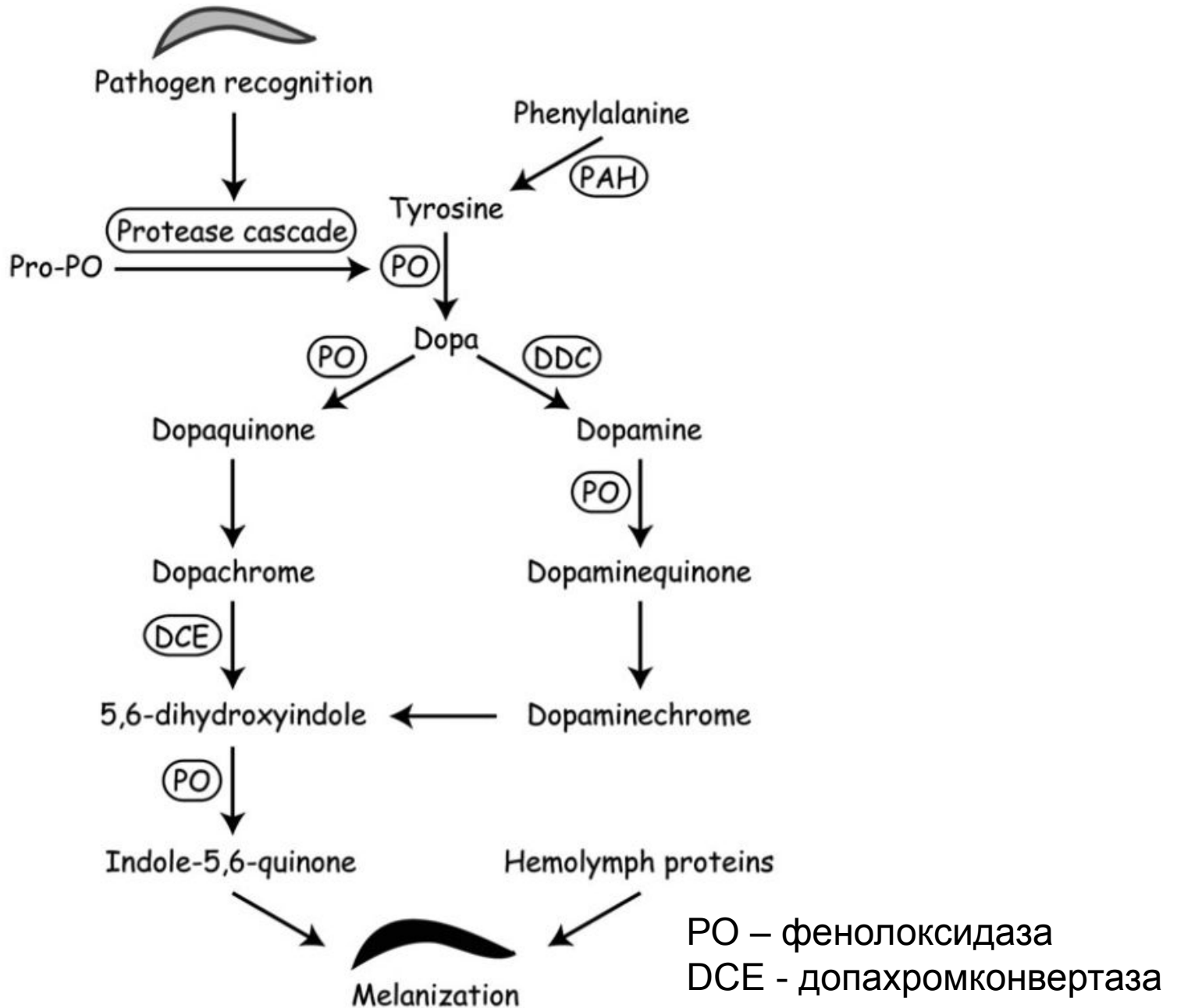


LIPOLYSACCHARIDE



Limulus polyphemus

СИНТЕЗ МЕЛАНИНА

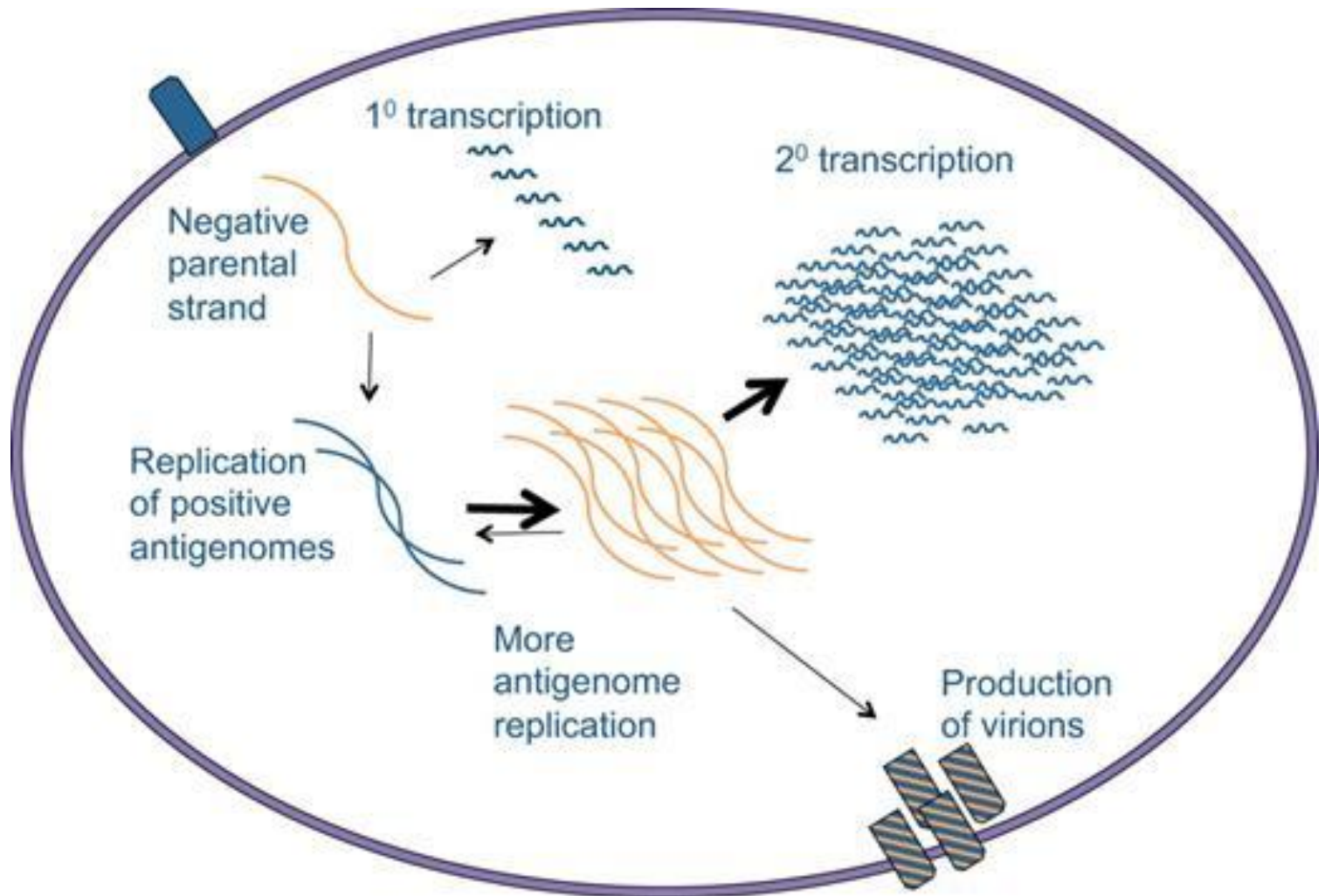


Вирусы членистоногих обычно имеют РНК-геном

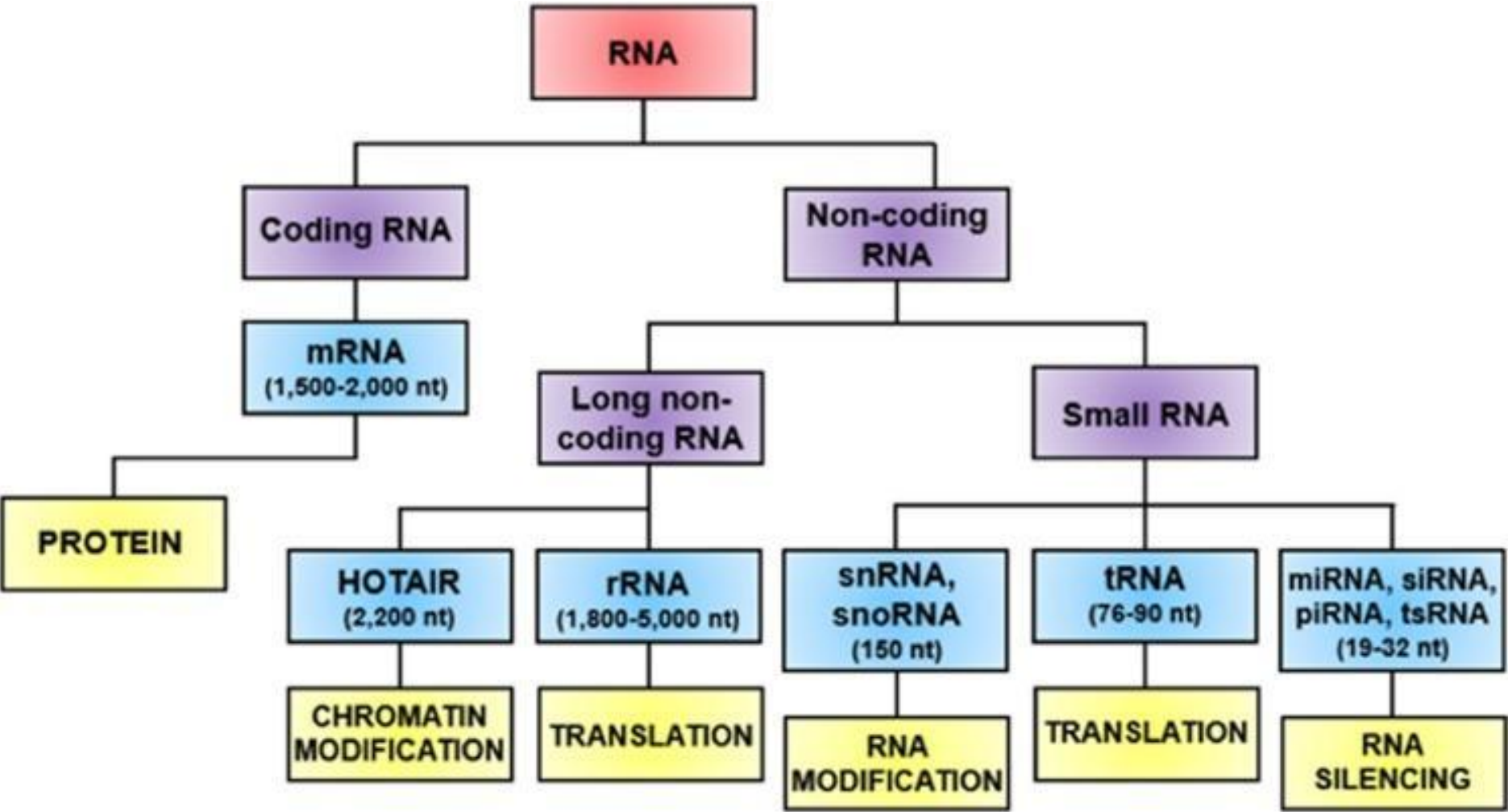
Virus-derived small RNA profiles of arthropod viruses. Modified from (van Mierlo et al., 2011).

Virus	Family	Host for study	Genome
<i>RNA viruses</i>			
Drosophila A virus	Tetraviridae	Drosophila ^b	(+)ssRNA
Drosophila C virus	Dicistroviridae	Drosophila ^b	(+)ssRNA
<i>Homalodisca coagulata</i> virus-1	Dicistroviridae	<i>Homalodisca vitripennis</i> ^a	(+)ssRNA
Noravirus	Unassigned	Drosophila ^b	(+)ssRNA
Sindbis virus	Alphaviridae	<i>Aedes aegypti</i> ^a	(+)ssRNA
Mosquito nodavirus	Nodaviridae	<i>Aedes aegypti</i> ^a	(+)ssRNA
West Nile virus	Flaviviridae	<i>Culex pipiens quinquefasciatus</i> ^a	(+)ssRNA
Semliki Forest virus	Alphaviridae	<i>Aedes albopictus</i> ^b and <i>Aedes aegypti</i> ^b	(+)ssRNA
Dengue virus –Serotype 2	Flaviviridae	<i>Aedes aegypti</i> ^{a,b}	(+)ssRNA
Cell fusing agent virus	Flaviviridae	<i>Aedes aegypti</i> ^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
<i>Homalodisca vitripennis</i> reovirus	Reoviridae	<i>Homalodisca vitripennis</i> ^a	dsRNA
Mosquito X virus	Unassigned	<i>Culex pipiens molestus</i> ^a , <i>Culex tritaeniorhynchus</i> ^a , <i>Aedes sinensis</i> ^a	Bipartite dsRNA
<i>DNA viruses</i>			
<i>Helicoverpa armigera</i> single nucleopolyhedrovirus	Baculoviridae	<i>Helicoverpa armigera</i> ^a	dsDNA
Vaccinia virus	Poxviridae	Drosophila ^b	dsDNA
Invertebrate iridescent virus 6	Iridoviridae	Drosophila ^{a,b}	dsDNA

Цикл репликации вируса дрозофилы DmelSV

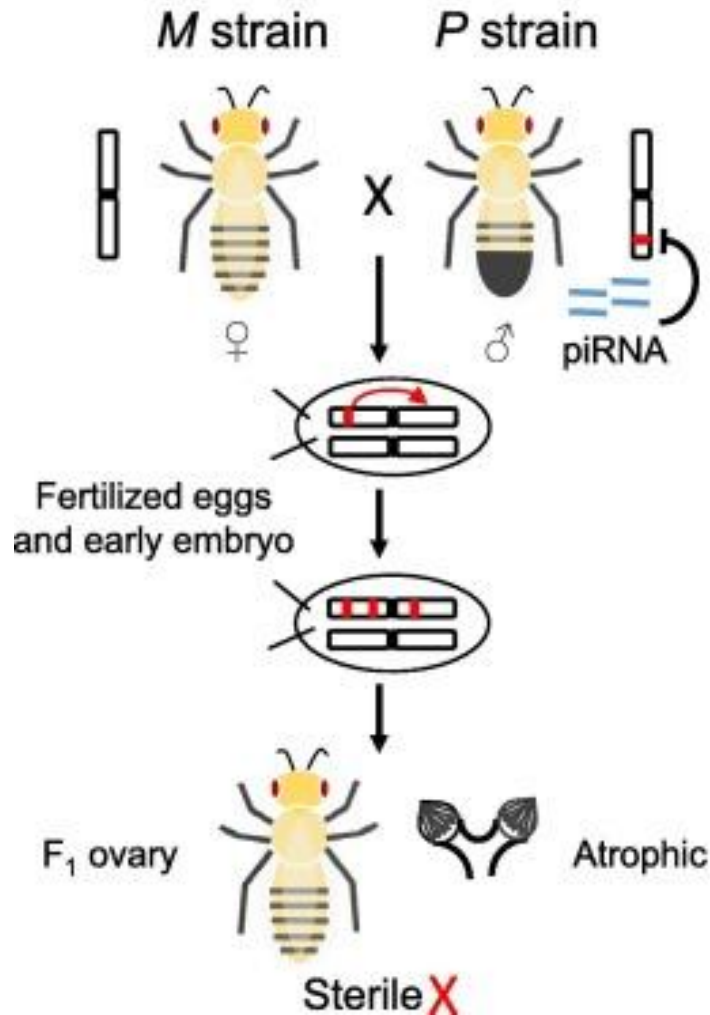


Классификация видов РНК эукариот

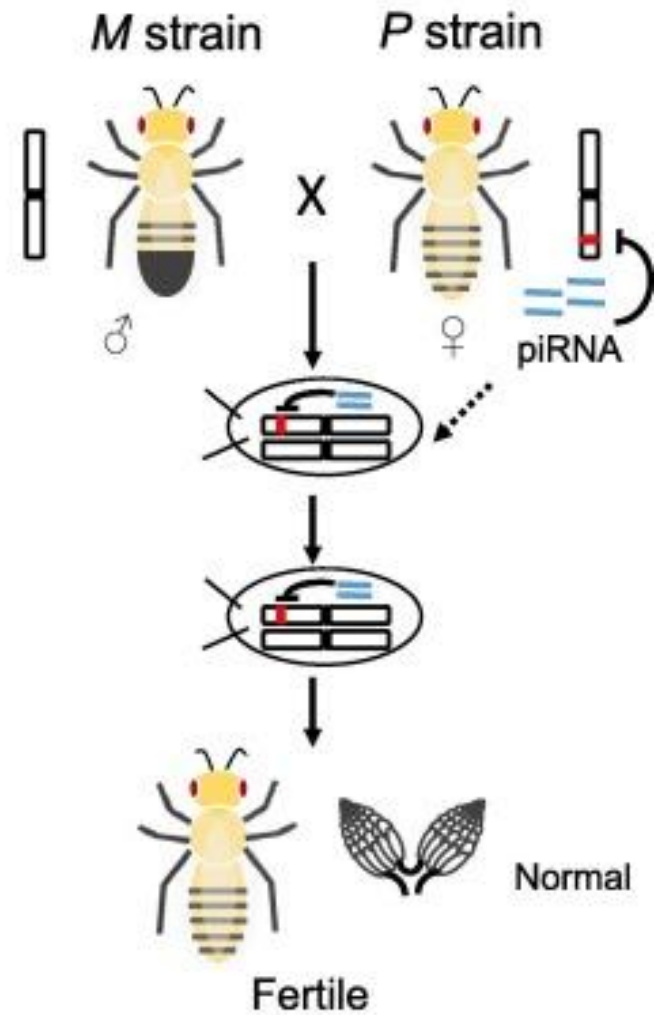


Гибридный дисгенез у дрозофилы

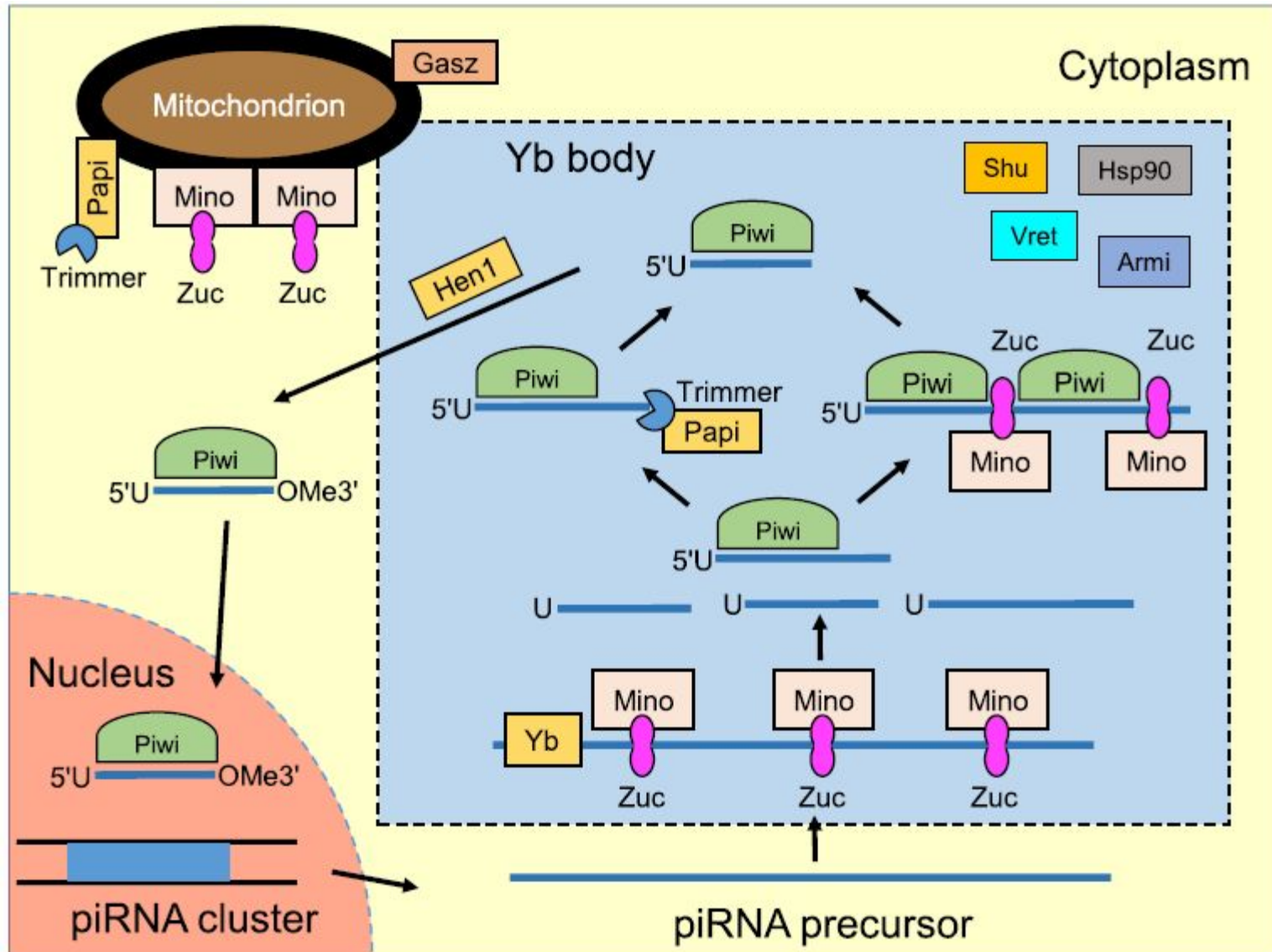
A Dysgenic cross



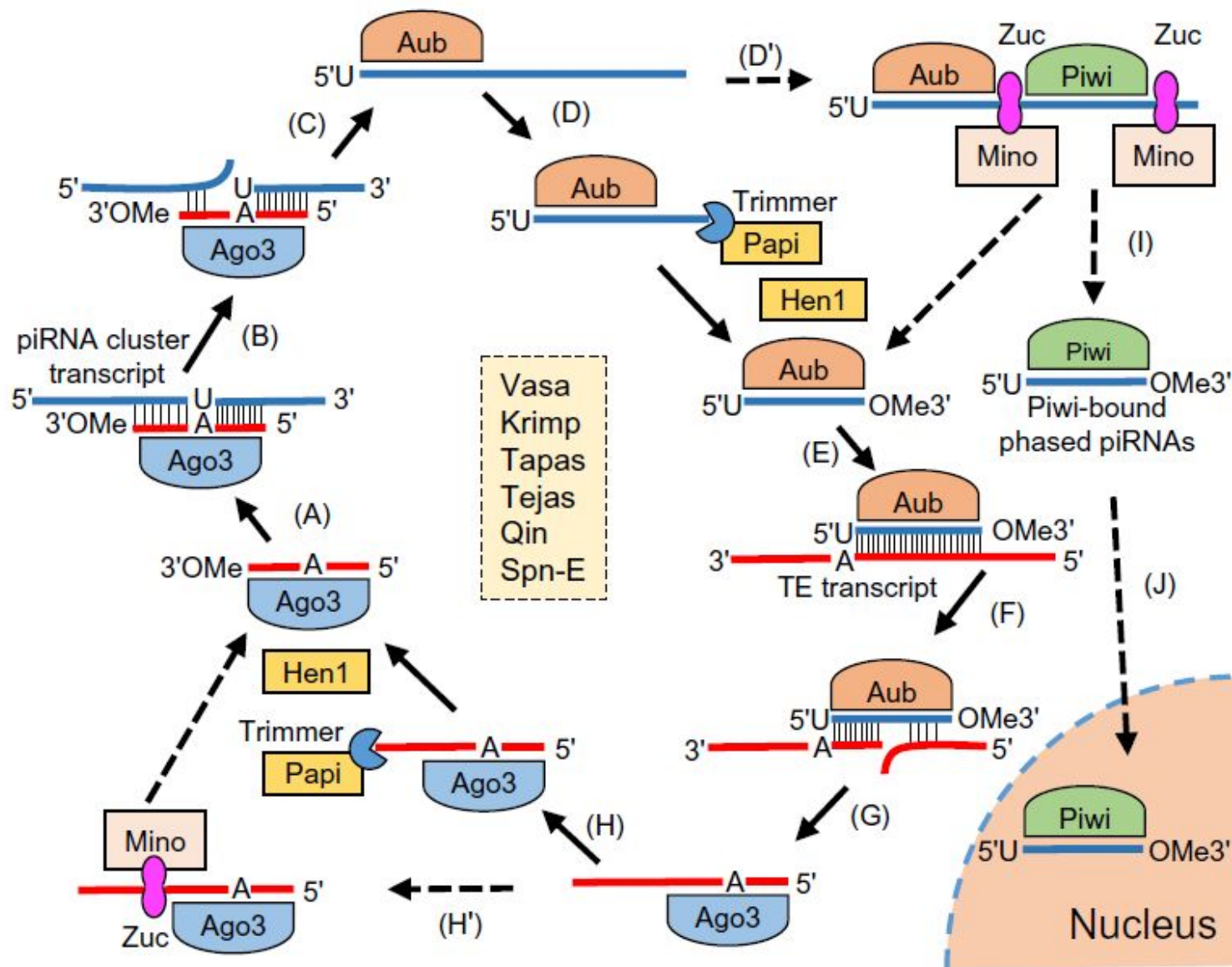
B Reciprocal cross



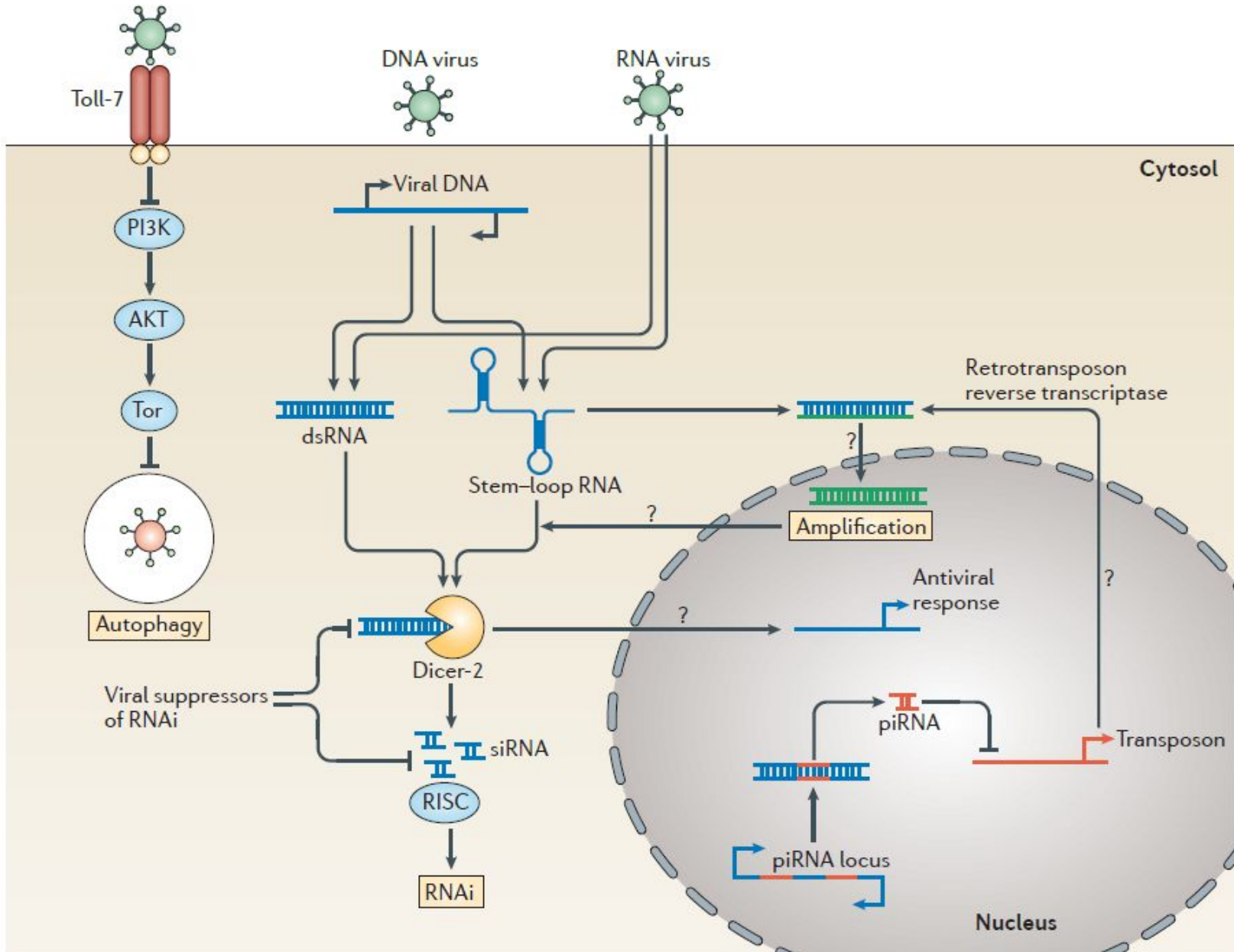
Первичный процессинг пиРНК



«Игра в пинг-понг» и амплификация piРНК

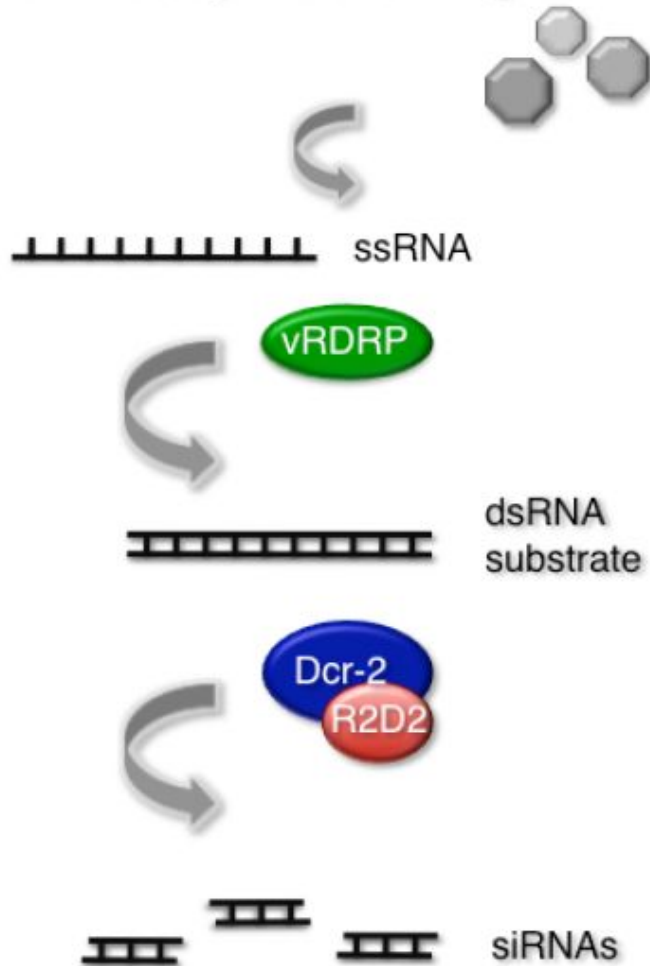


Противовирусный иммунитет дрозофилы



Принципиальная схема РНК-интерференции у дрозофилы

Structure dependent dicing



Sequence specific slicing

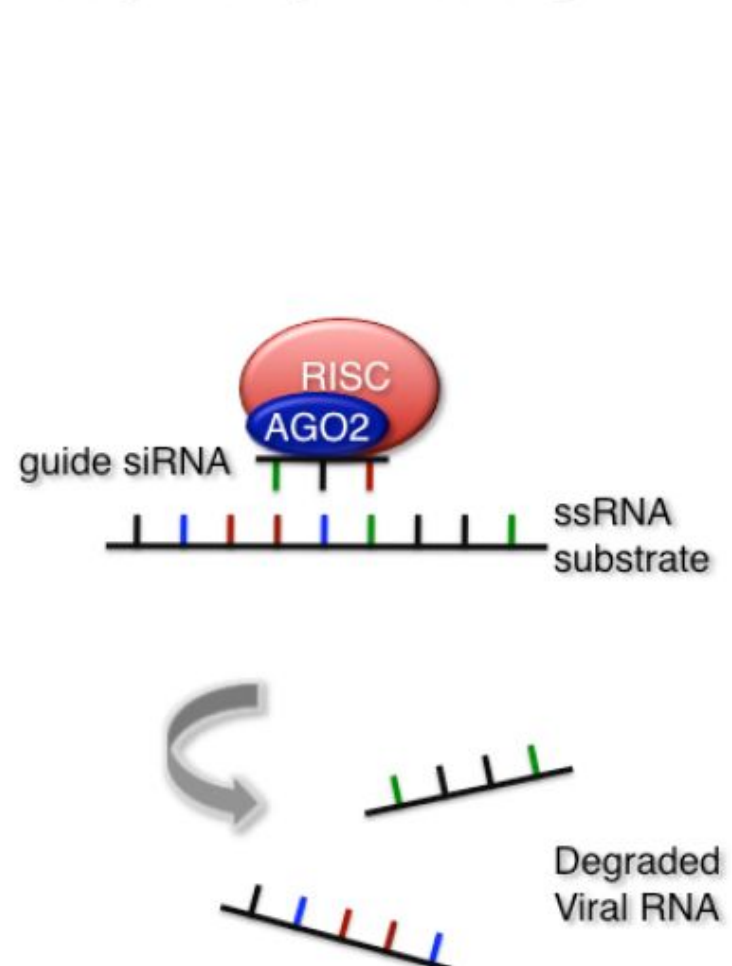
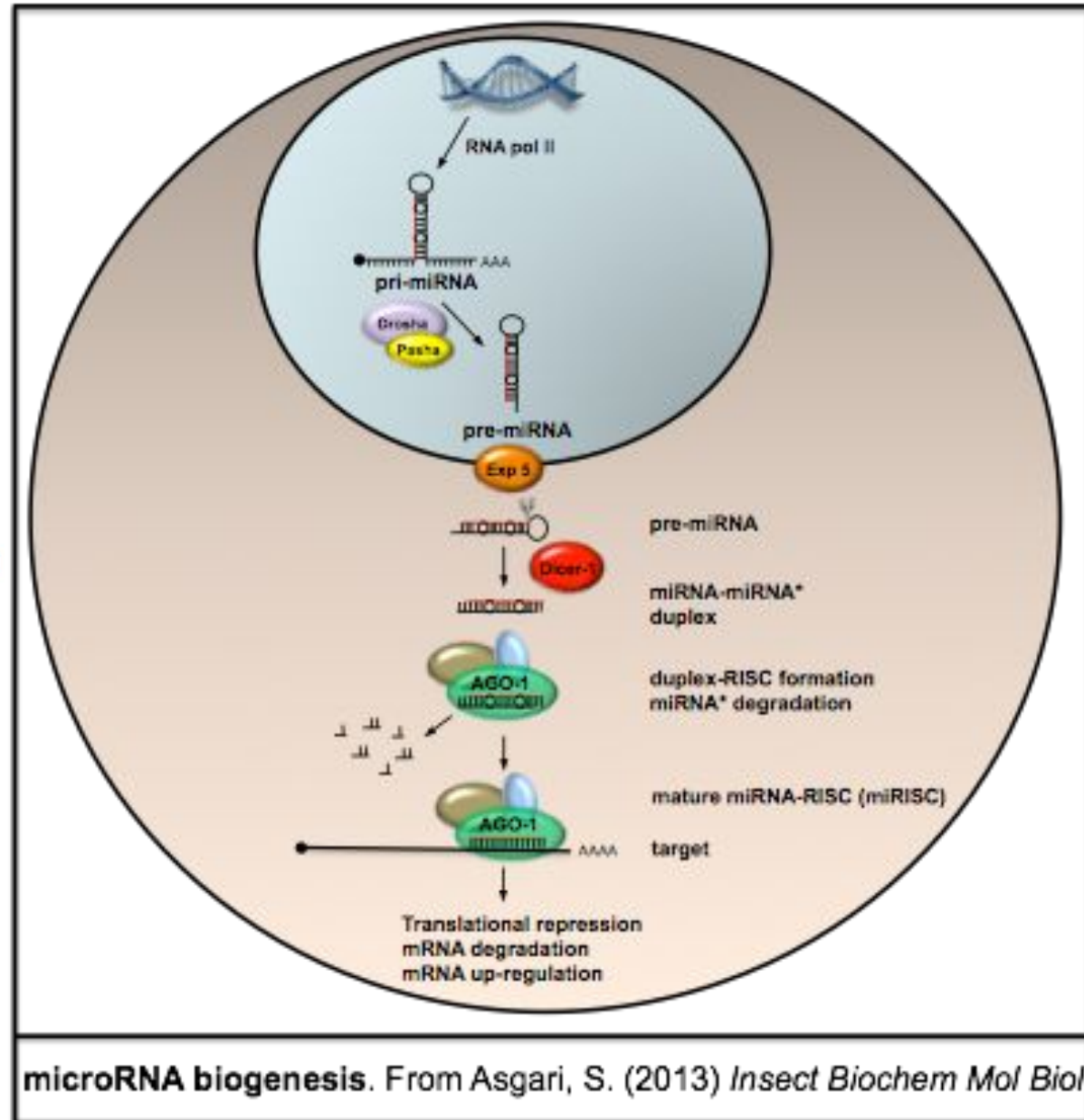
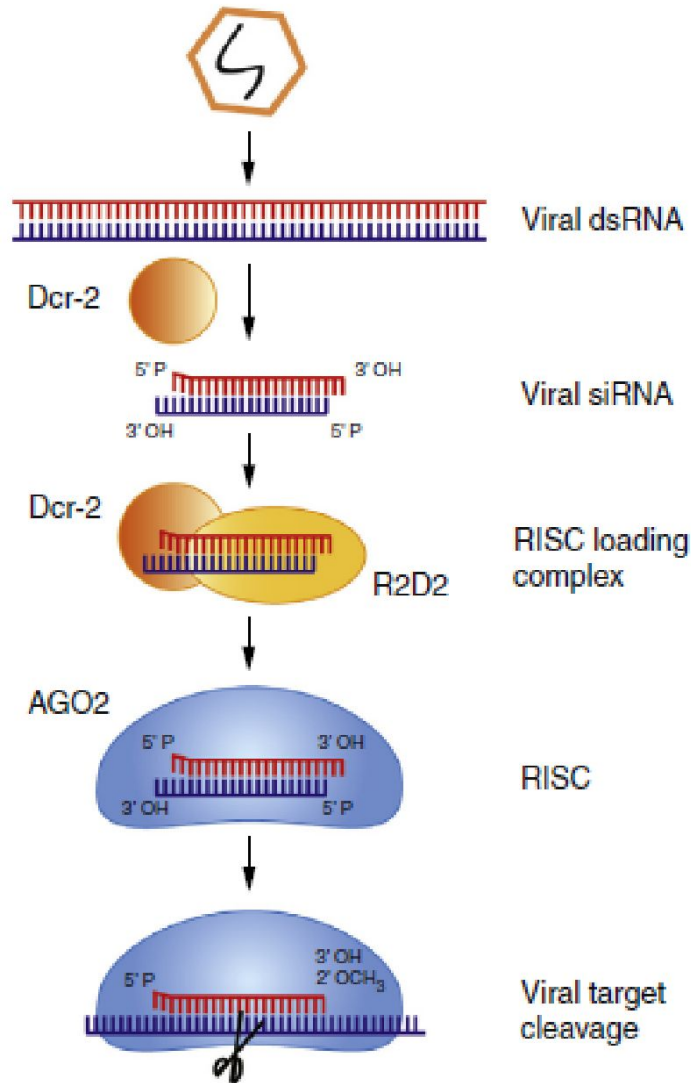
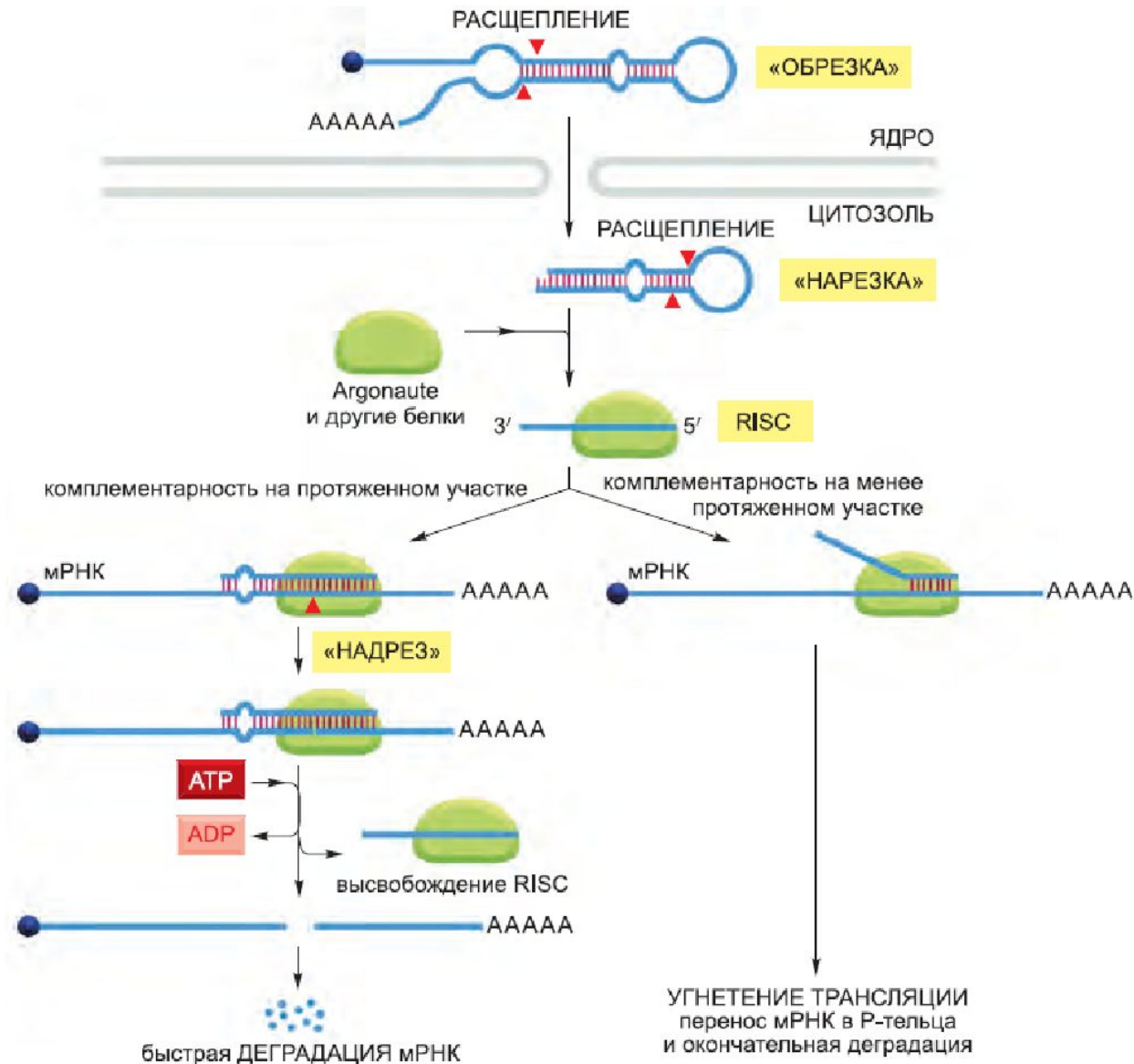


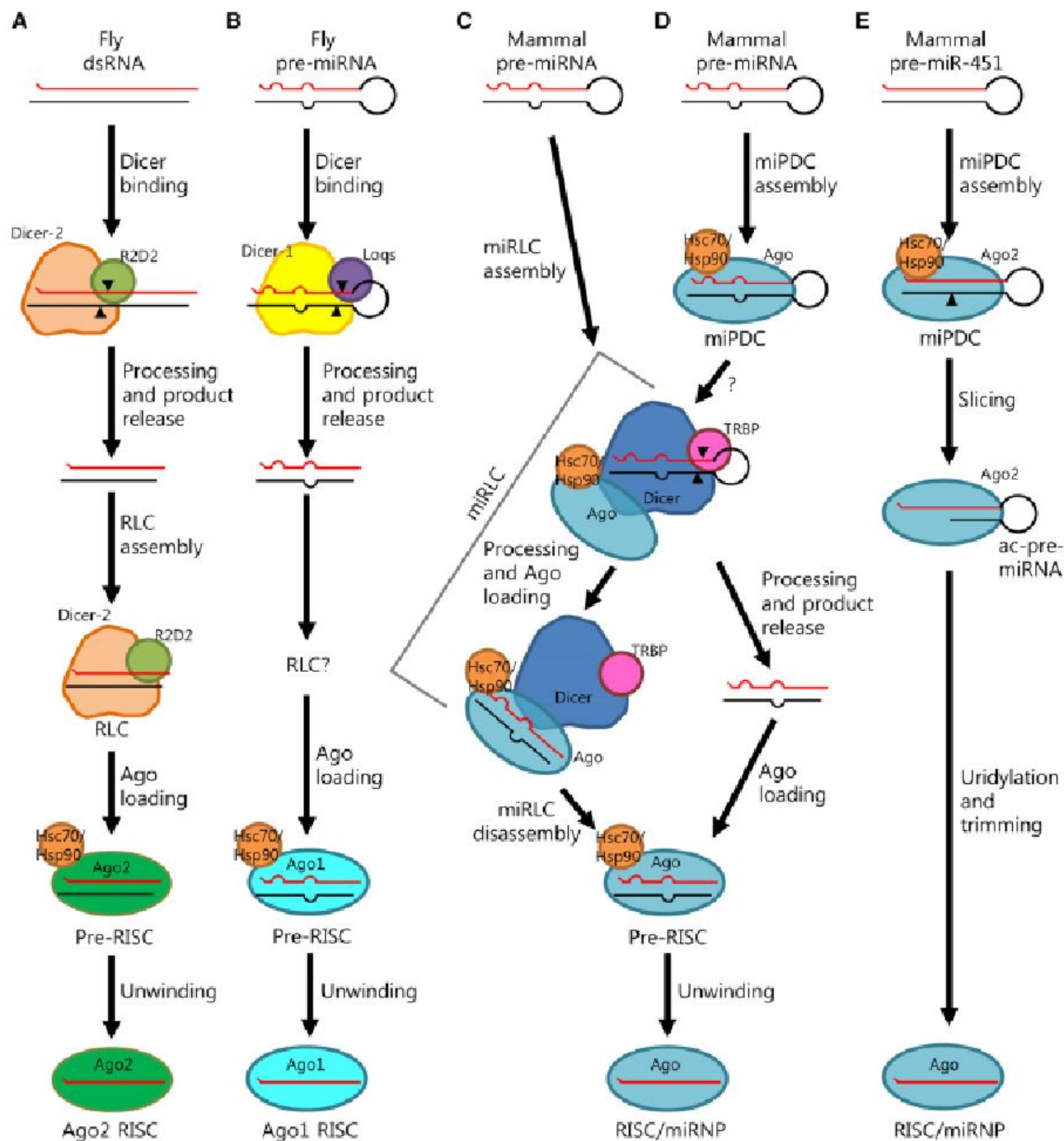
Схема образования miRNA и siRNA у дрозофилы



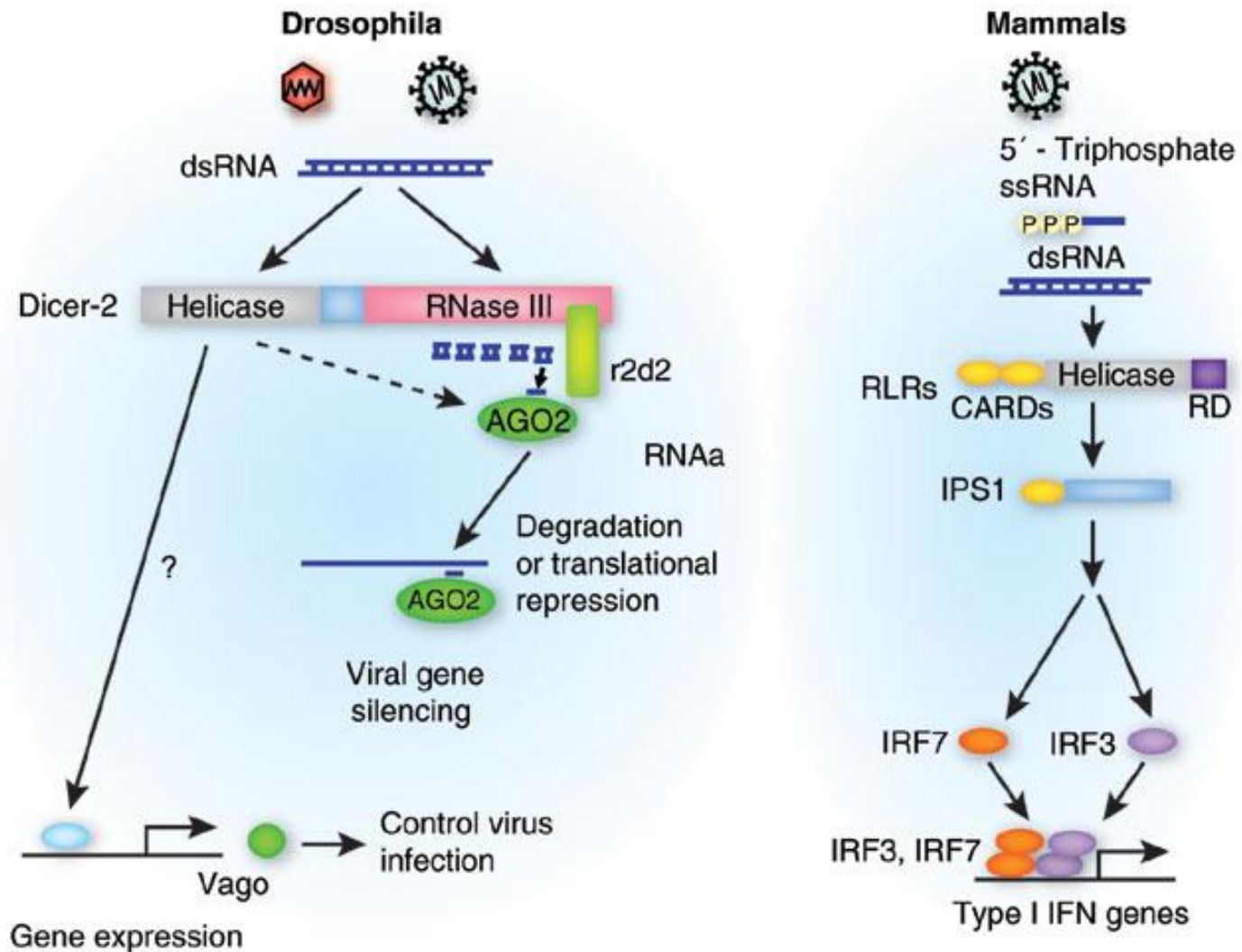
microRNA biogenesis. From Asgari, S. (2013) *Insect Biochem Mol Biol*

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

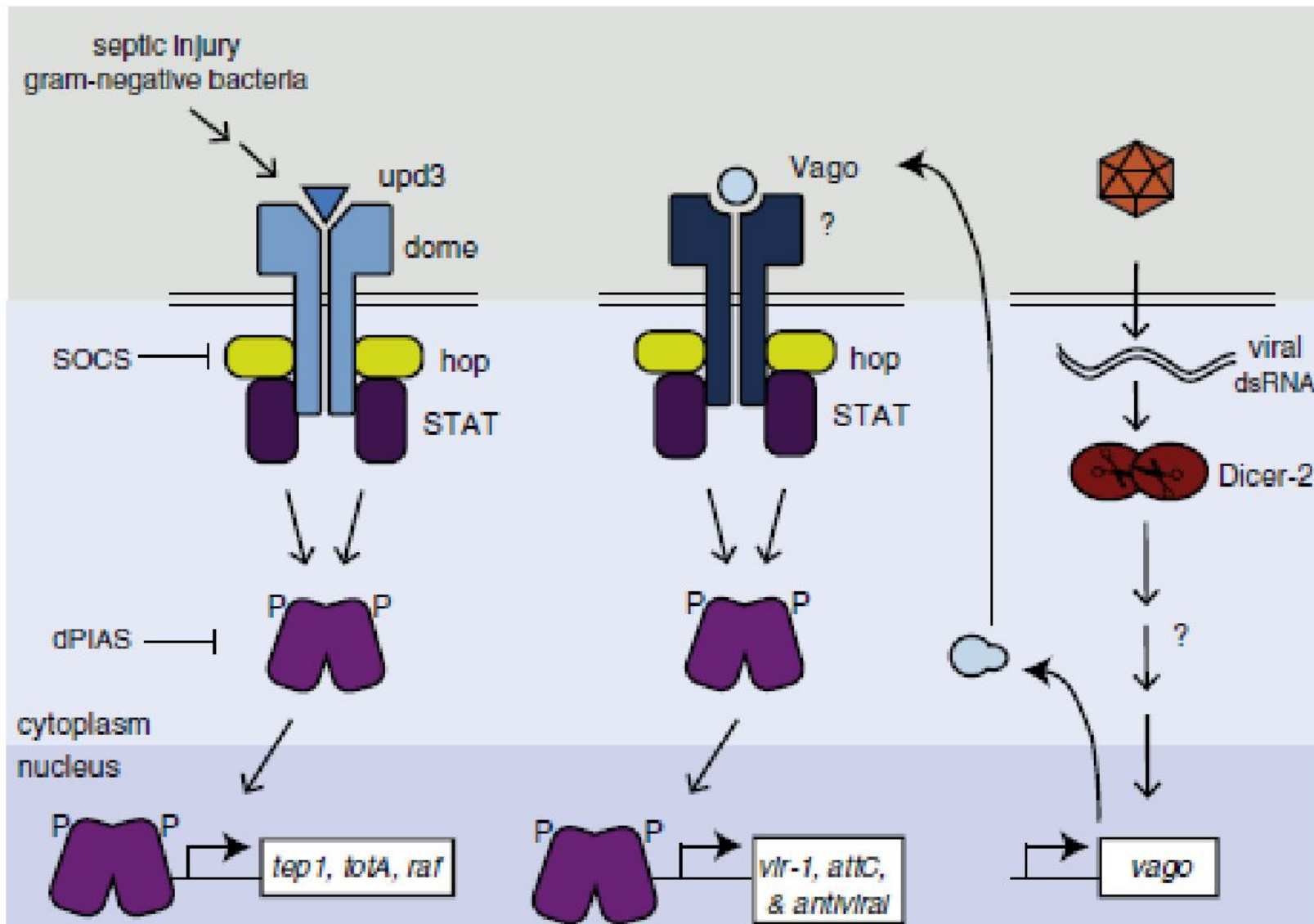




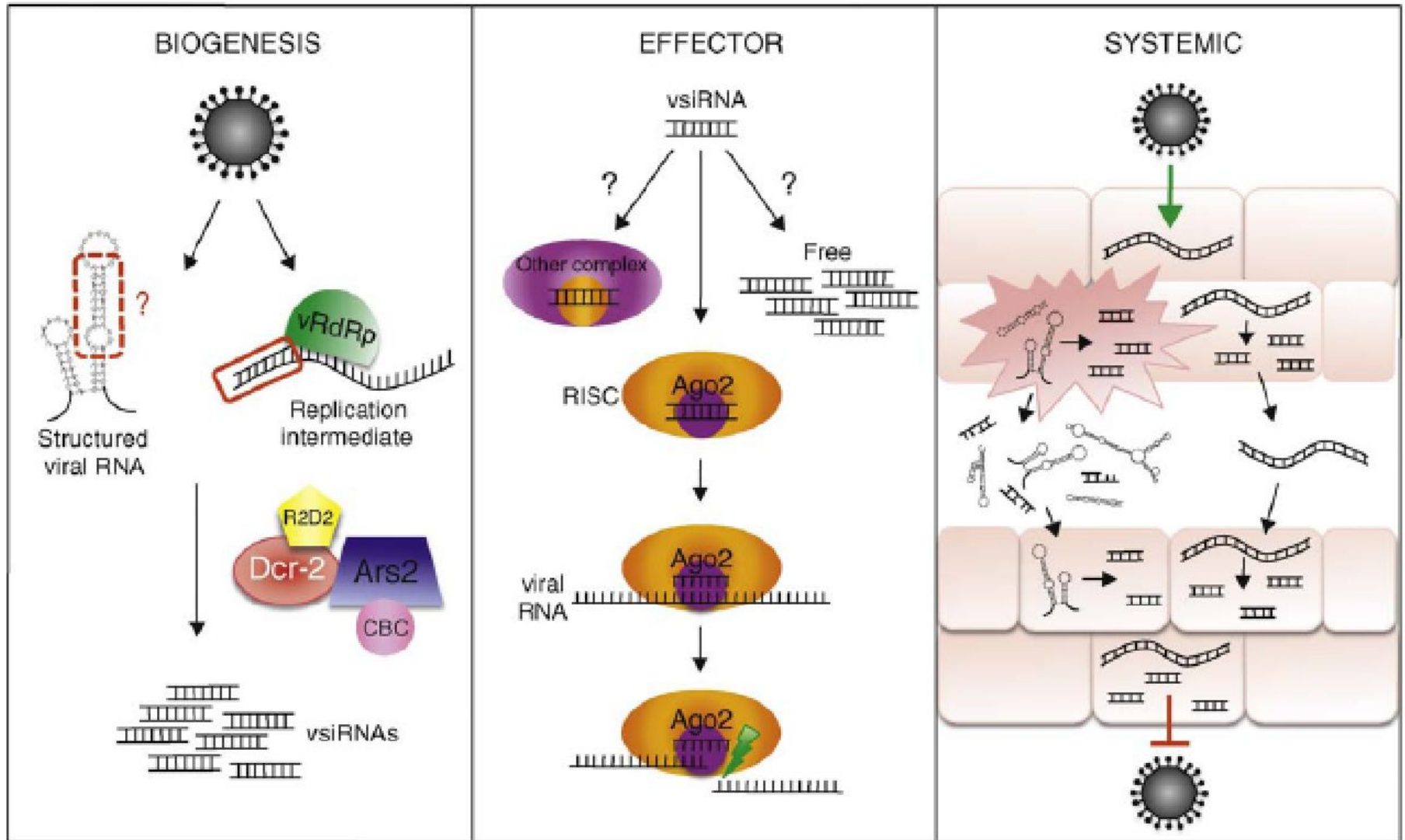
Различия между RNAi дрозодилы и RLR-сигналингом млекопитающих



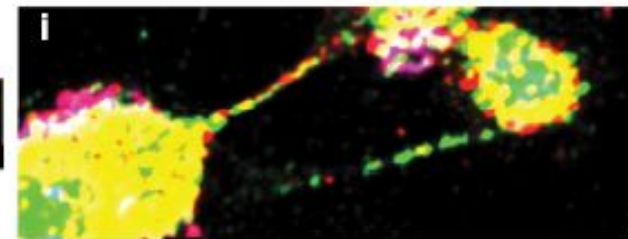
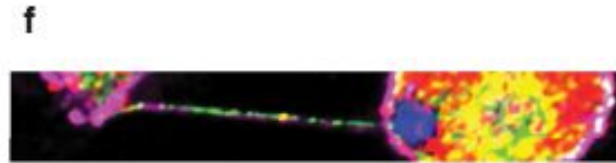
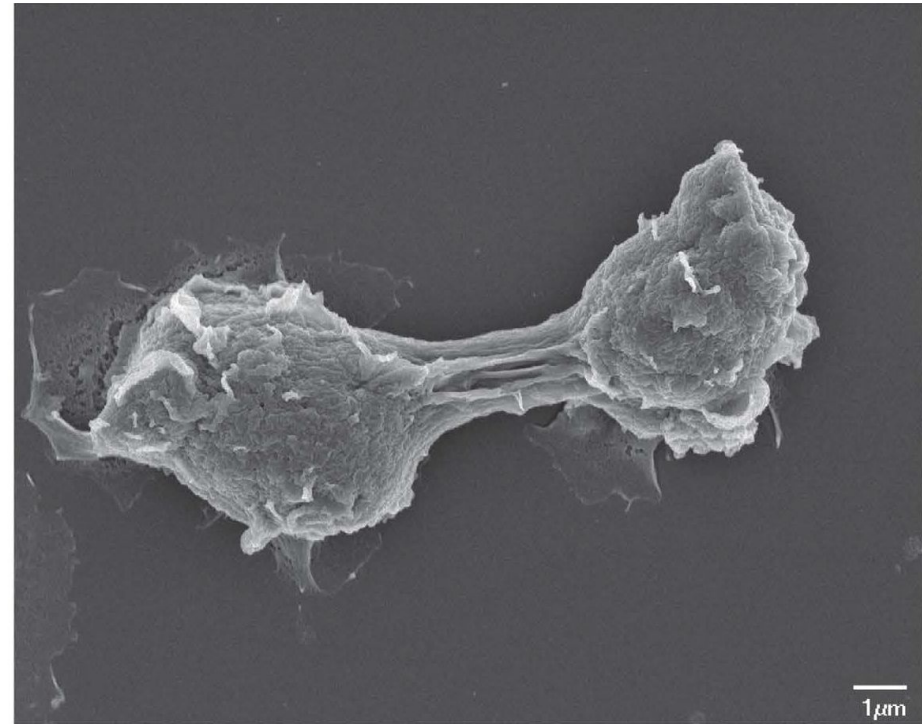
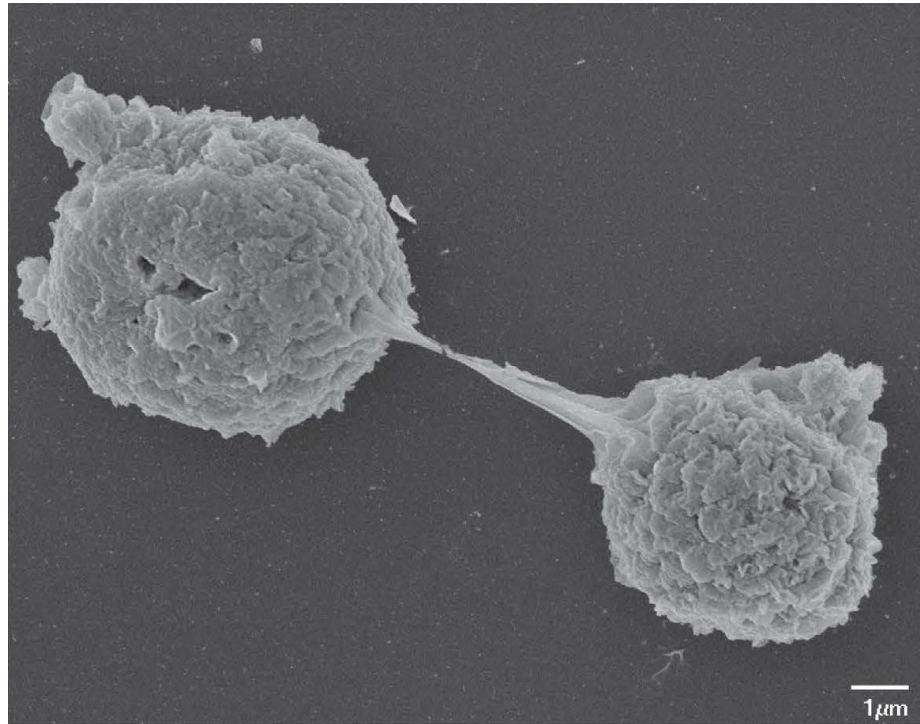
Vago и STAT-сигналинг



Системное действие РНК-интерференции у мух



Клетки дрозофилы передают друг другу RNAi комплексы по нанотрубкам

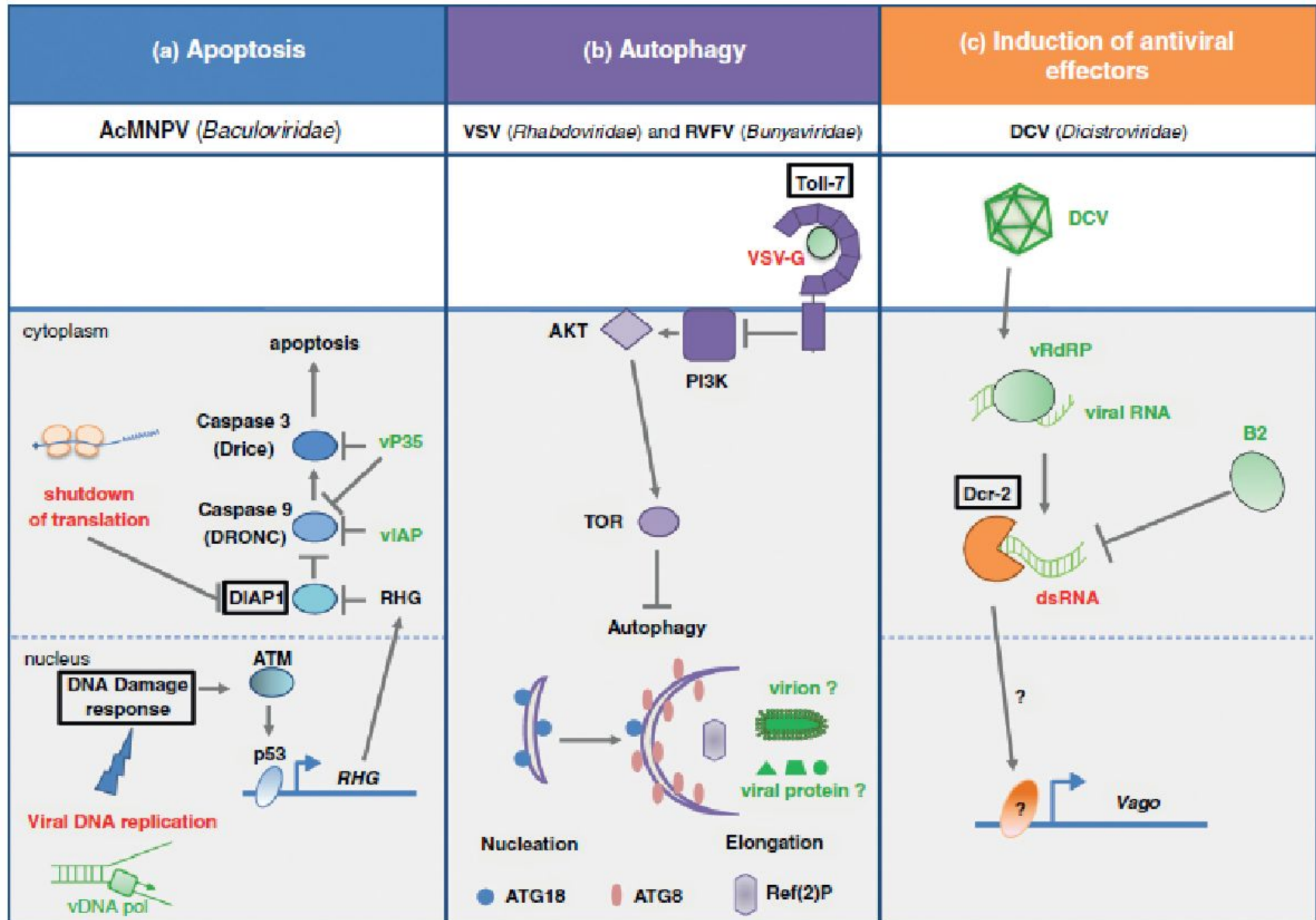


Rab7 / Ago2 / DAPI / Phalloidin

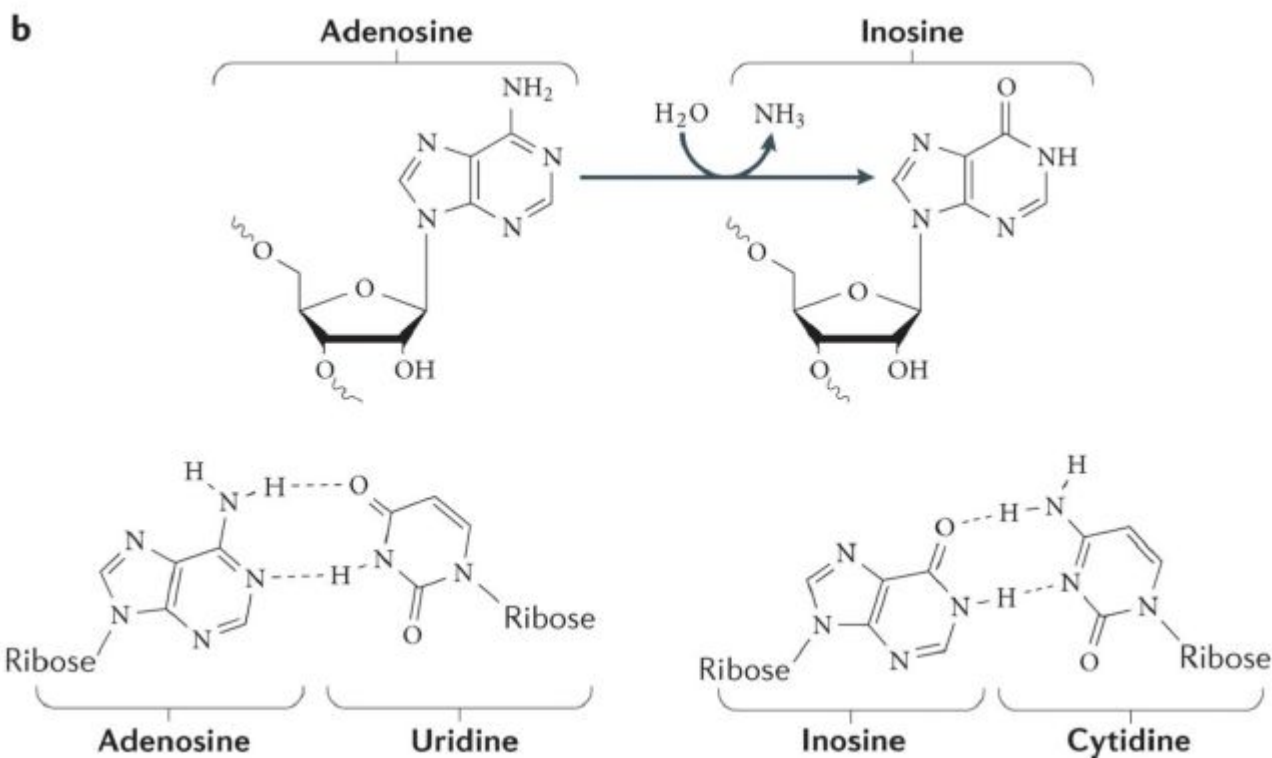
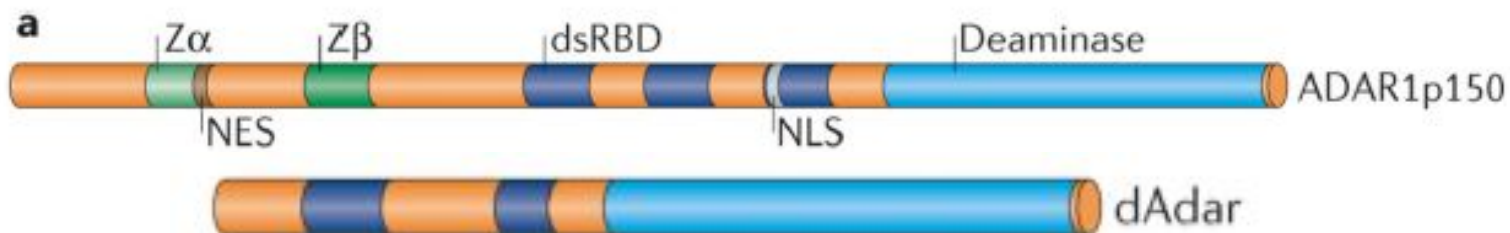
dsRNA / Ago2 / DAPI / Phalloidin

CG4572 / Ago2 / DAPI / Phalloidin

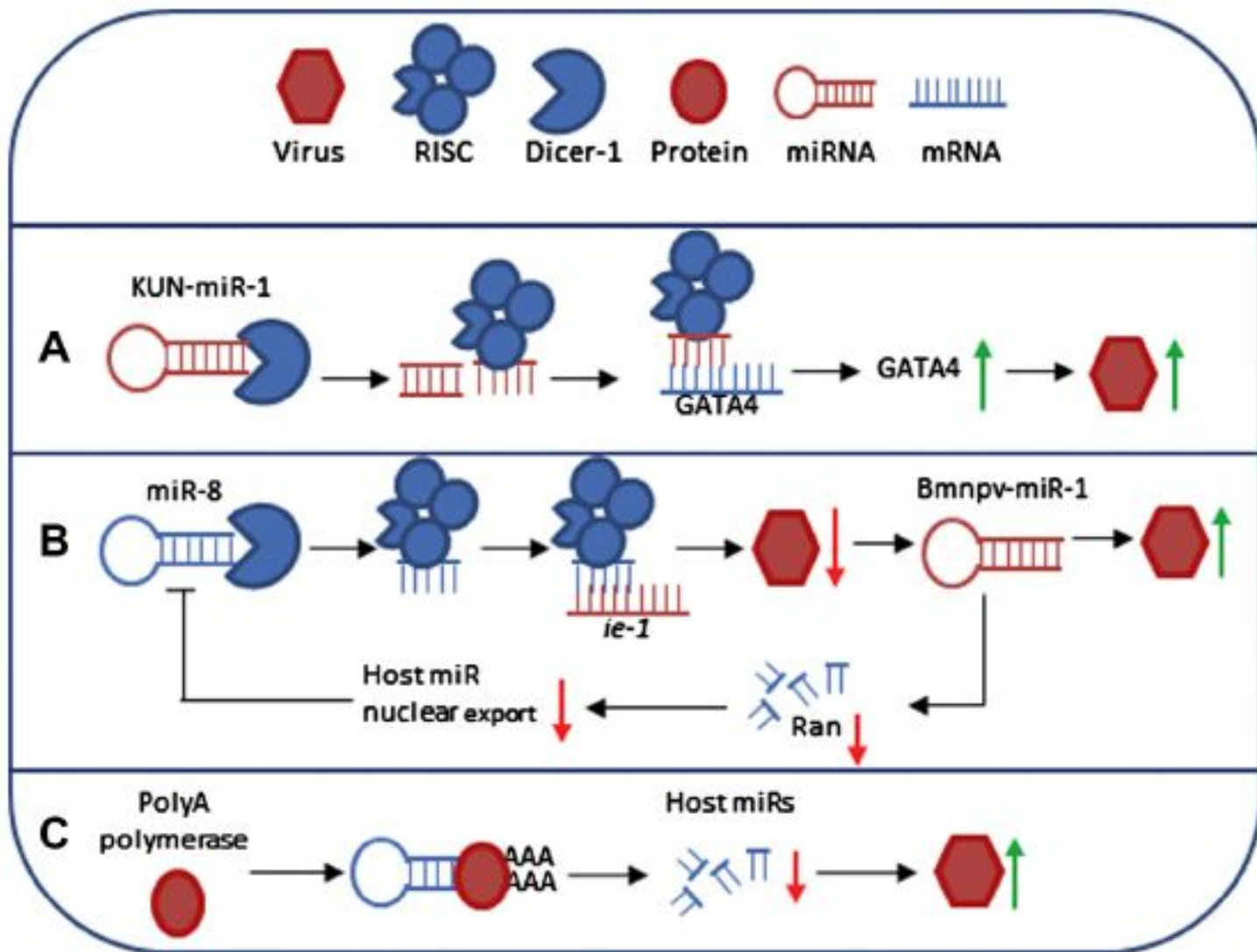
Варианты клеточного ответа на вирусную инфекцию у дрозофилы



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.

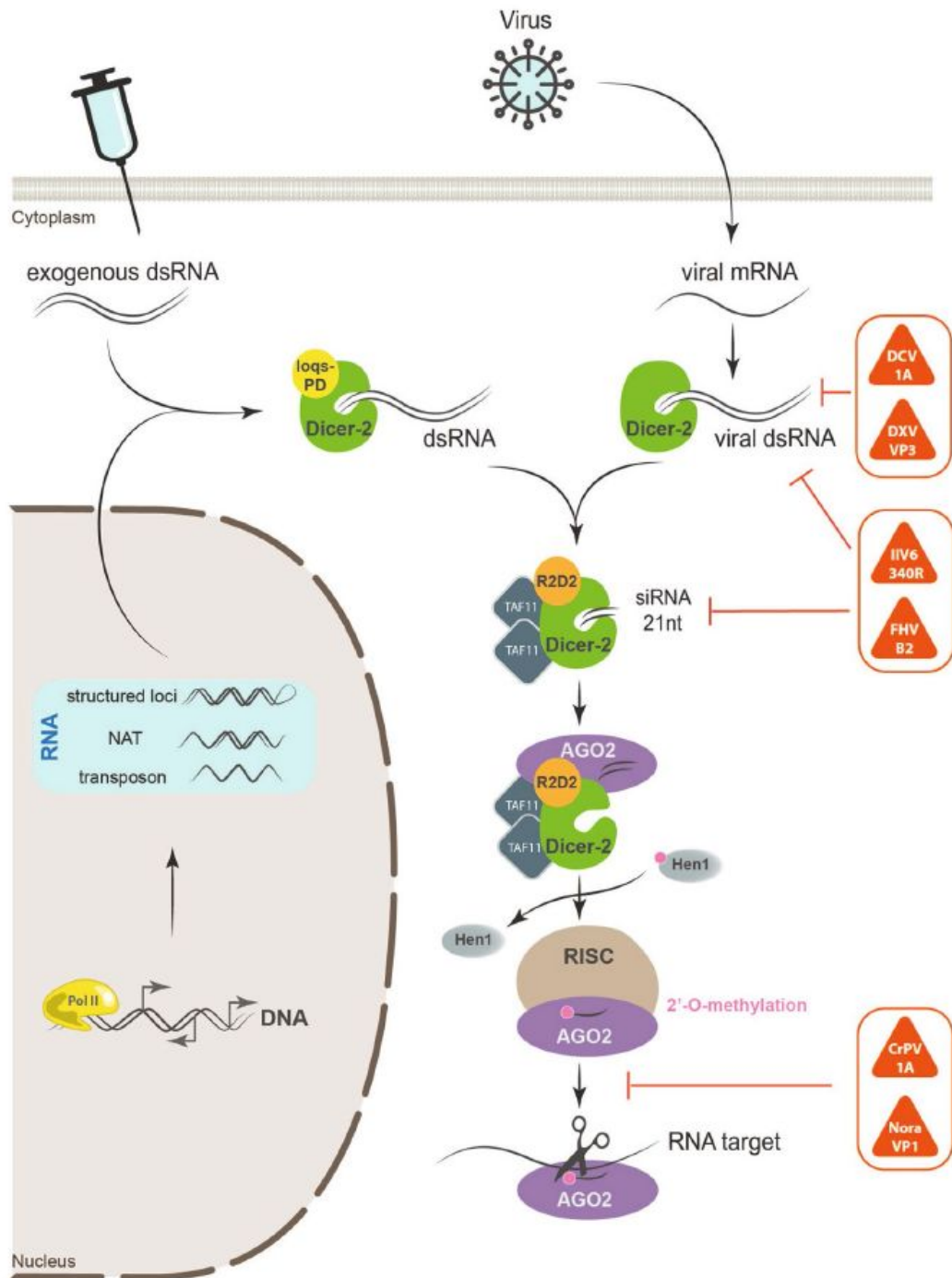
Virus/bacteria	Host insect	Insect factor	Virus factor	Mechanism/finding
Poxvirus (Vaccinia virus)	<i>Drosophila</i> DL1 cells	miR-34, miR-11, miR-184	Poly-A-Polymerase	Poly-A-polymerase poly adenylates miRs to lead to degradation
	<i>Drosophila</i> fat body	miR-8		miR-8 decreases drosomycin and dipteracin AMPs in fat body, independent of PI3 K
<i>Wolbachia</i>	<i>Aedes aegypti</i>	Aae-miR-2940	Unknown	<i>Wolbachia</i> 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
<i>Bombyx mori</i> baculovirus	<i>B. mori</i>	Exportin-5 miRNA export	Bmnpv-miR-1	Viral miRNA leads to RNAi repression of host Ran cofactor of exportin 5
Nudivirus (HzNV-1)	<i>Spodoptera frugiperda</i> IPLB-SF-21		<i>Pag1</i> miRNA	<i>Pag1</i> miRNA degrades viral early gene <i>hhi1</i> in latent infections

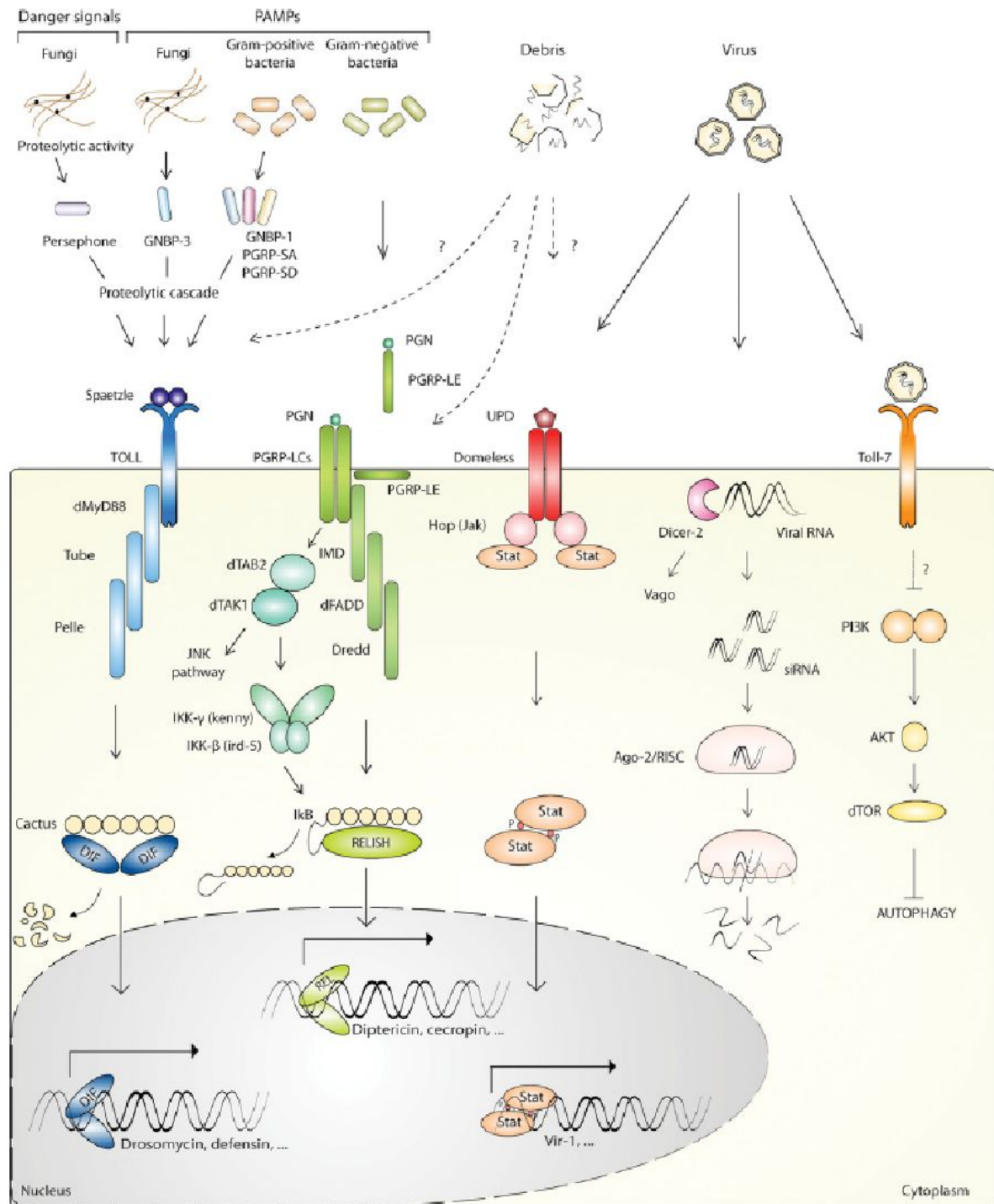
Arthropod virus-encoded protein or RNA suppressors of RNAi.

Virus	Antiviral suppressor	Mode of action
Flock house virus	B2 protein	Binds dsRNA and siRNAs
<i>Drosophila</i> 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 (<i>in vitro</i>)

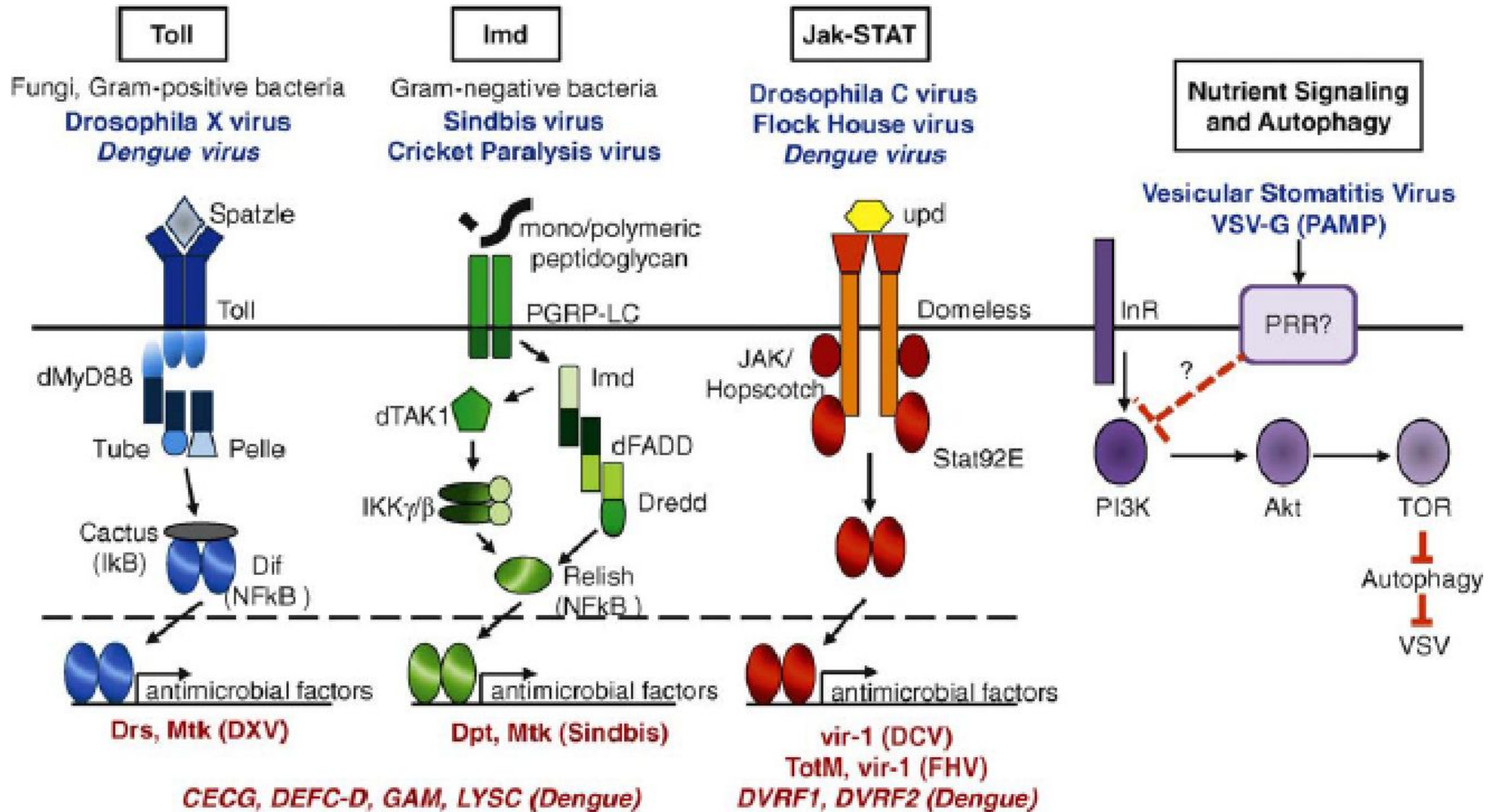
Viral RNAI suppressors in insect viruses and arboviruses

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





Вирусы дрозифилы и активируемые ими пути сигналинга

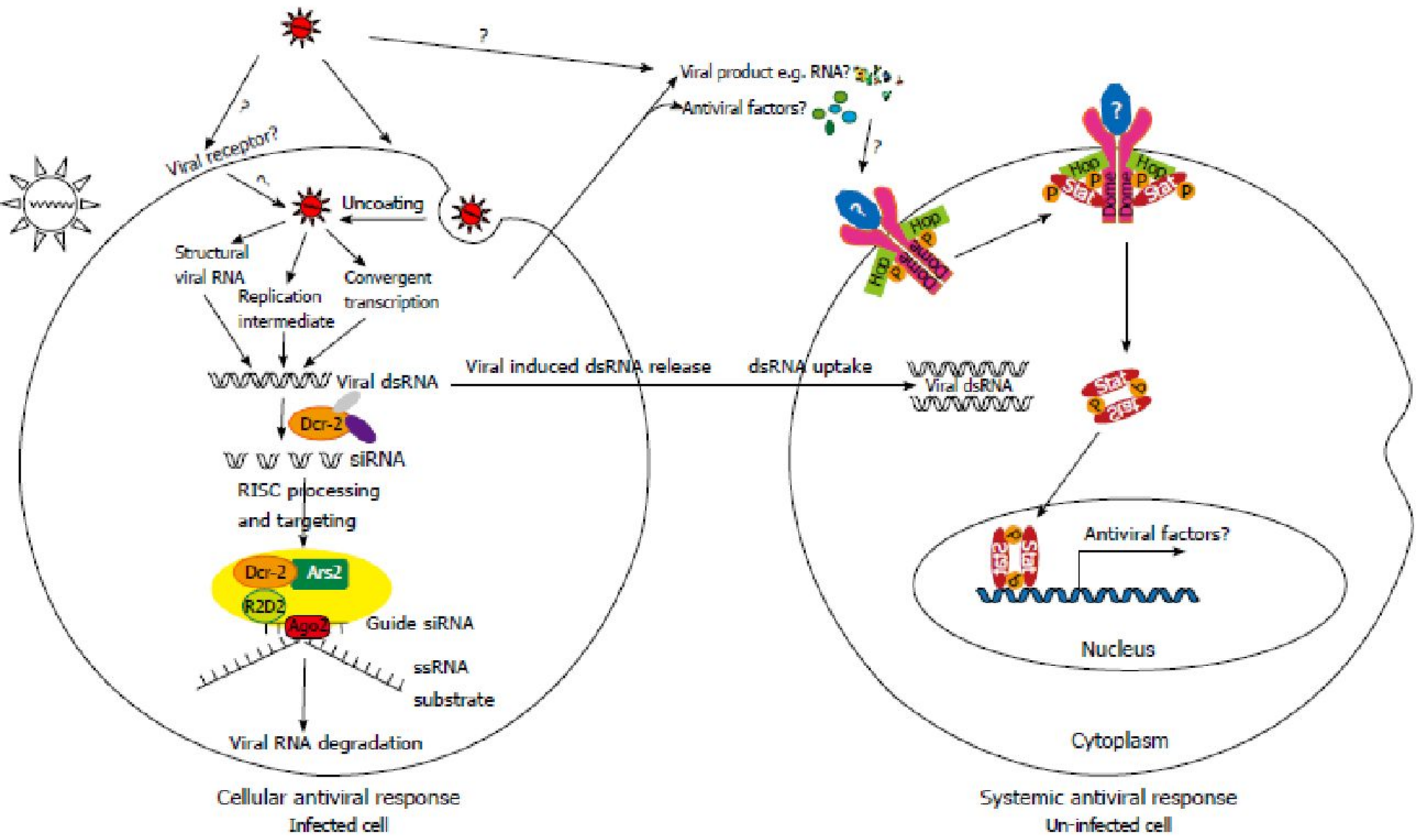


Против вирусов также могут быть использованы пути Toll и Imd

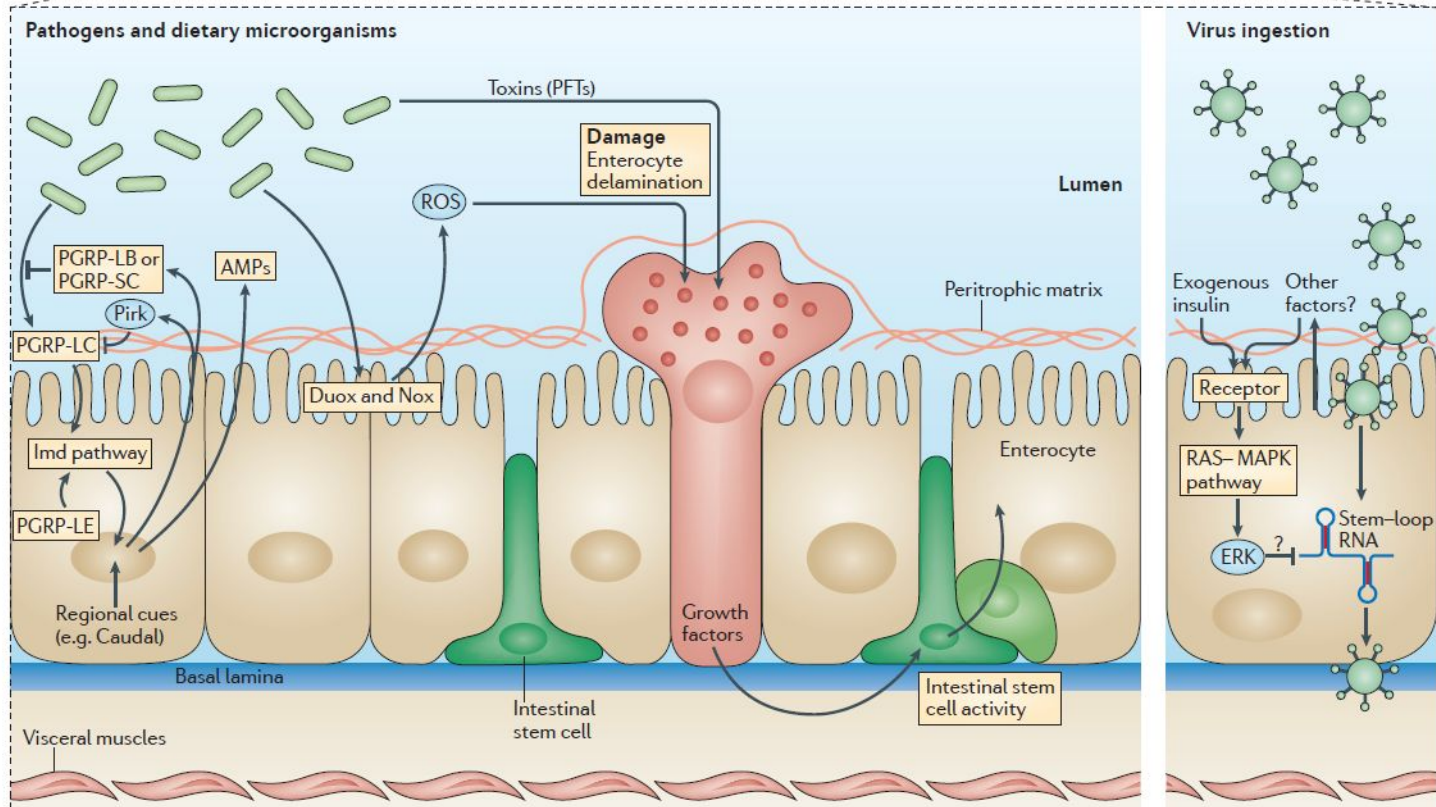
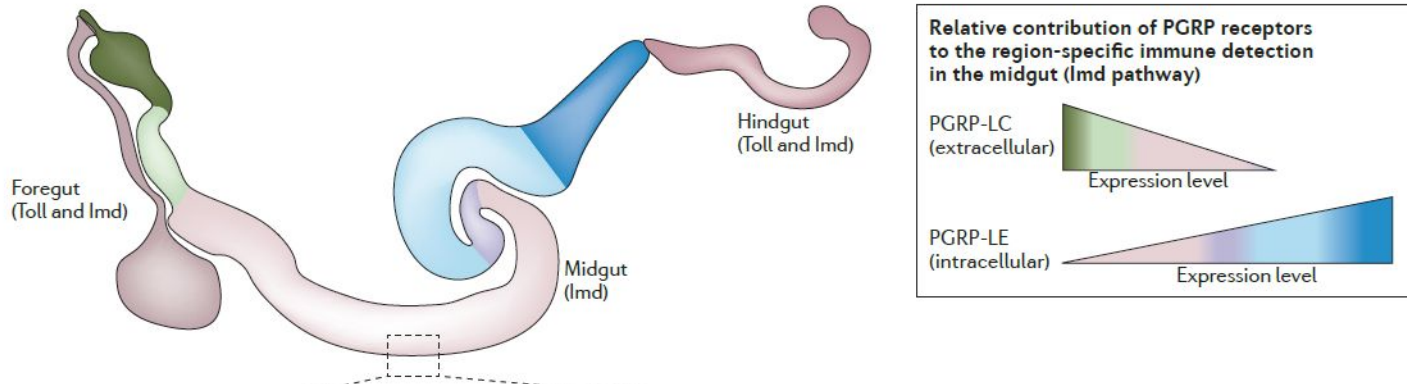
Inducible immune pathways implicated in antiviral defense in *Drosophila* and mosquito.

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

Механизмы противовирусной защиты – обобщение



Иммунитет кишечника мухи



Структура гена белка Dscam дрозофилы

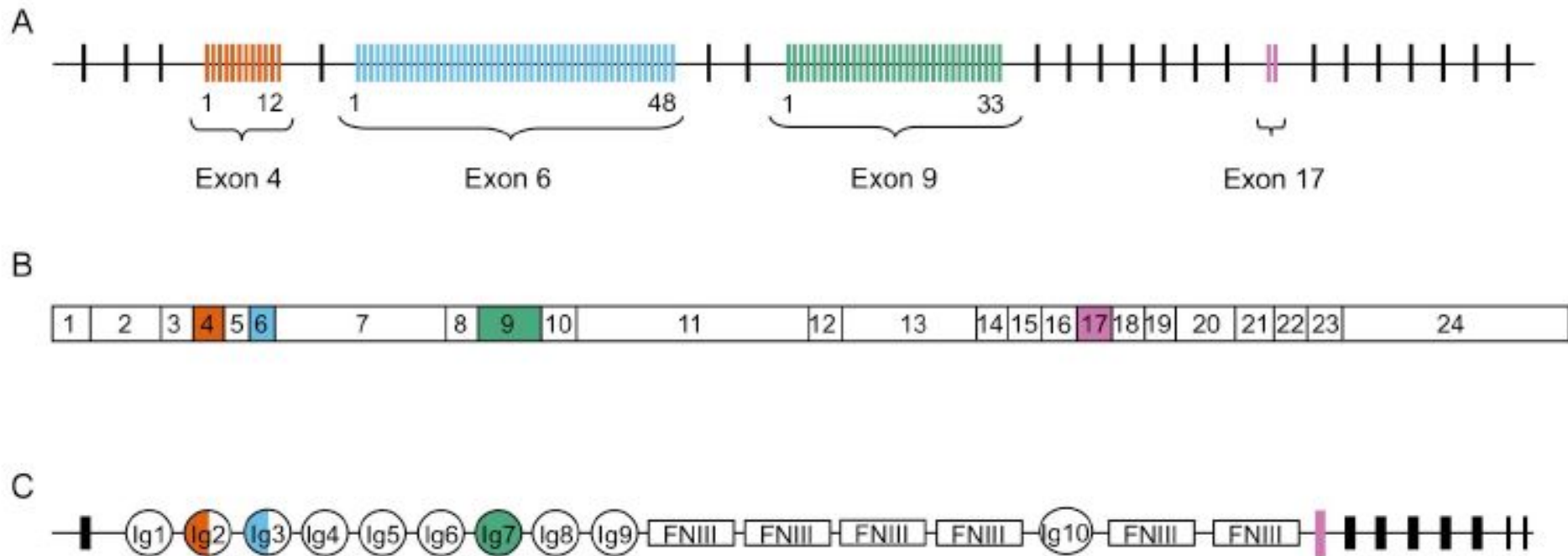
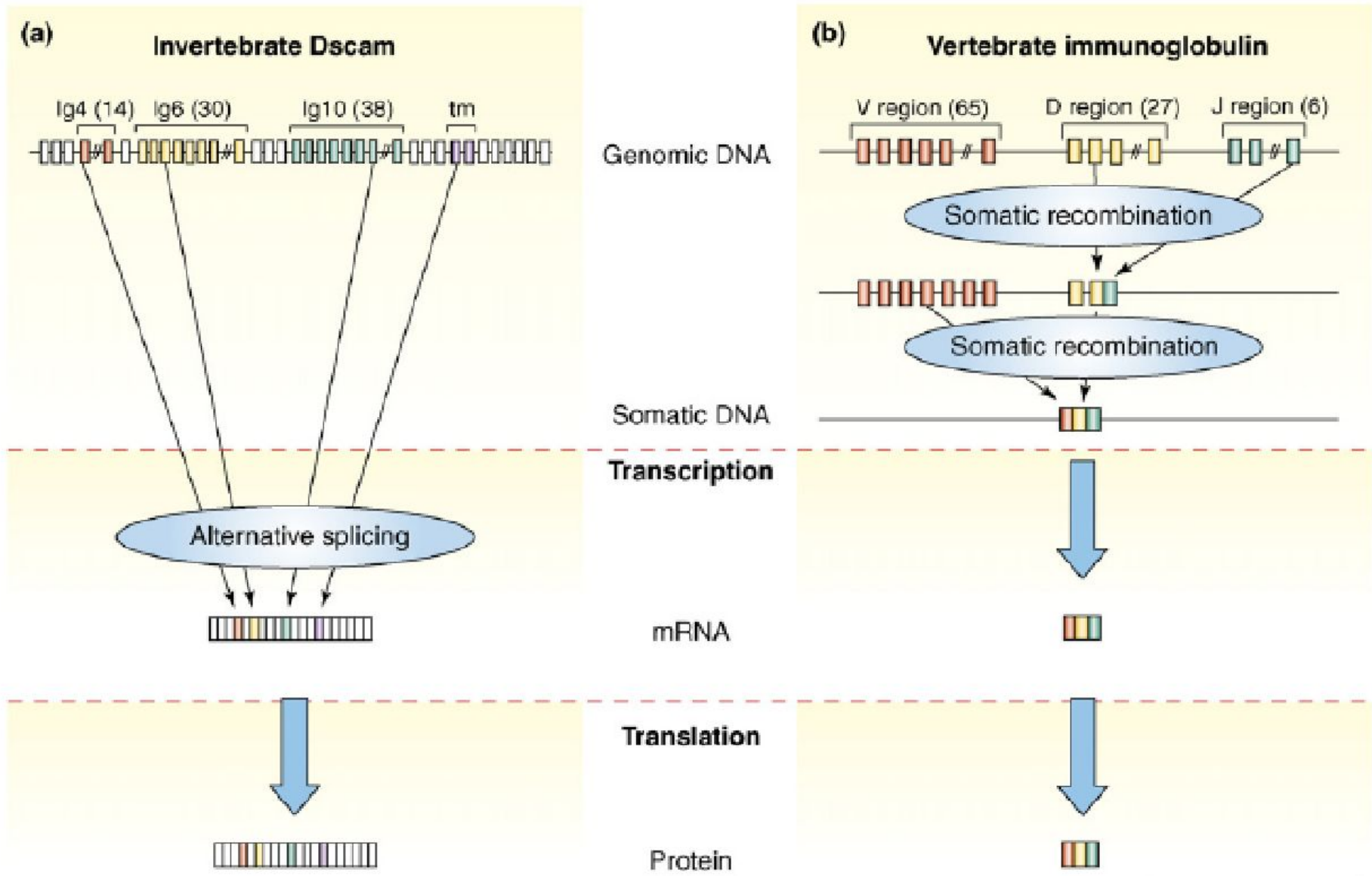
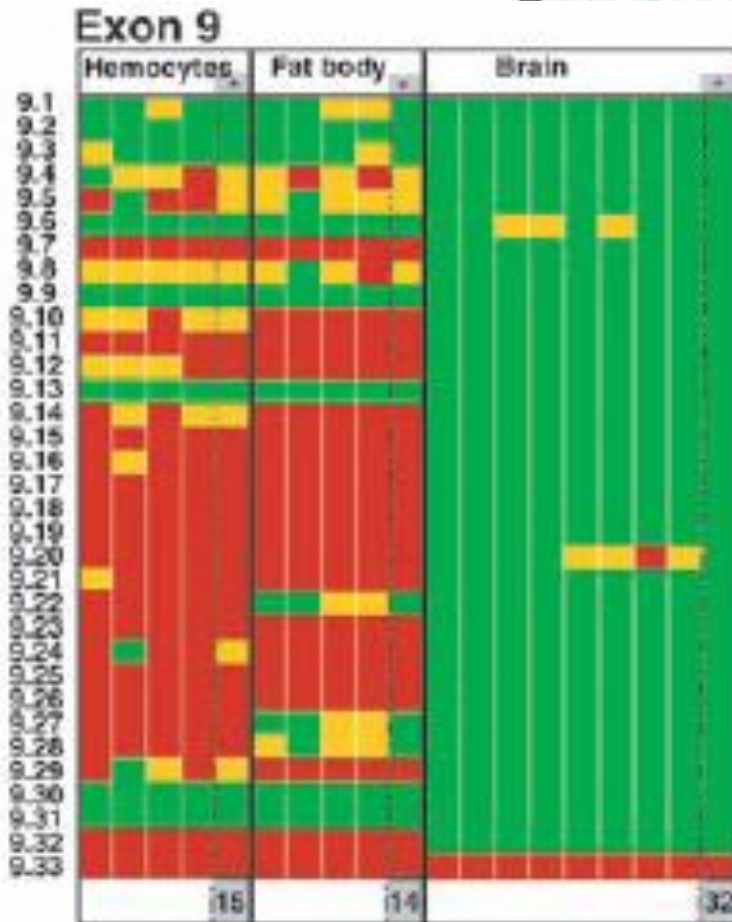
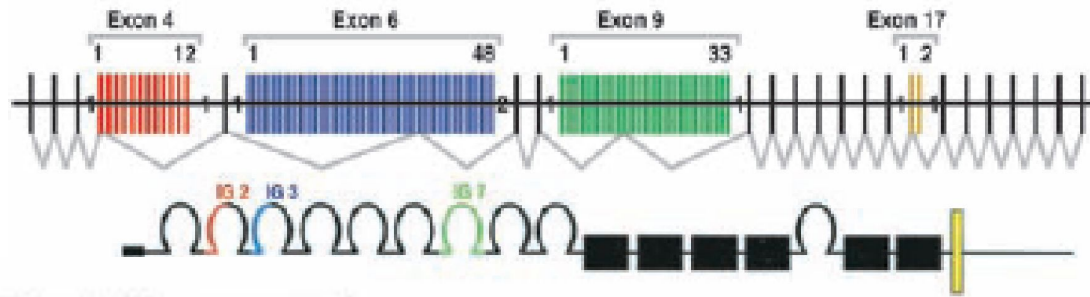


Figure 1 (A) *Dscam-hv* genomic DNA for *Drosophila melanogaster*. The gene consists of 20 constant exons (shown as black lines), mutually exclusive alternative splicing occurs for exons 4 (red lines), 6 (blue lines), 9 (green lines) and 17 (purple lines); one of 12 exon 4 alternatives, one of 48 exon 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 *Drasophila*); the N-terminal half of Ig3 (exon 6 in *Drasophila*), all of Ig7 (exon 9 in *Drasophila*), and the transmembrane domain (Exon 17 in *Drasophila* (figure after [6])).

Dscam и иммуноглобулины позвоночных



В иммунной системе мухи *Dscam* обладает дифференциальной экспрессией



Dietmar Schmucker

Что мы можем интерпретировать в пользу роли Dscam в иммунитете

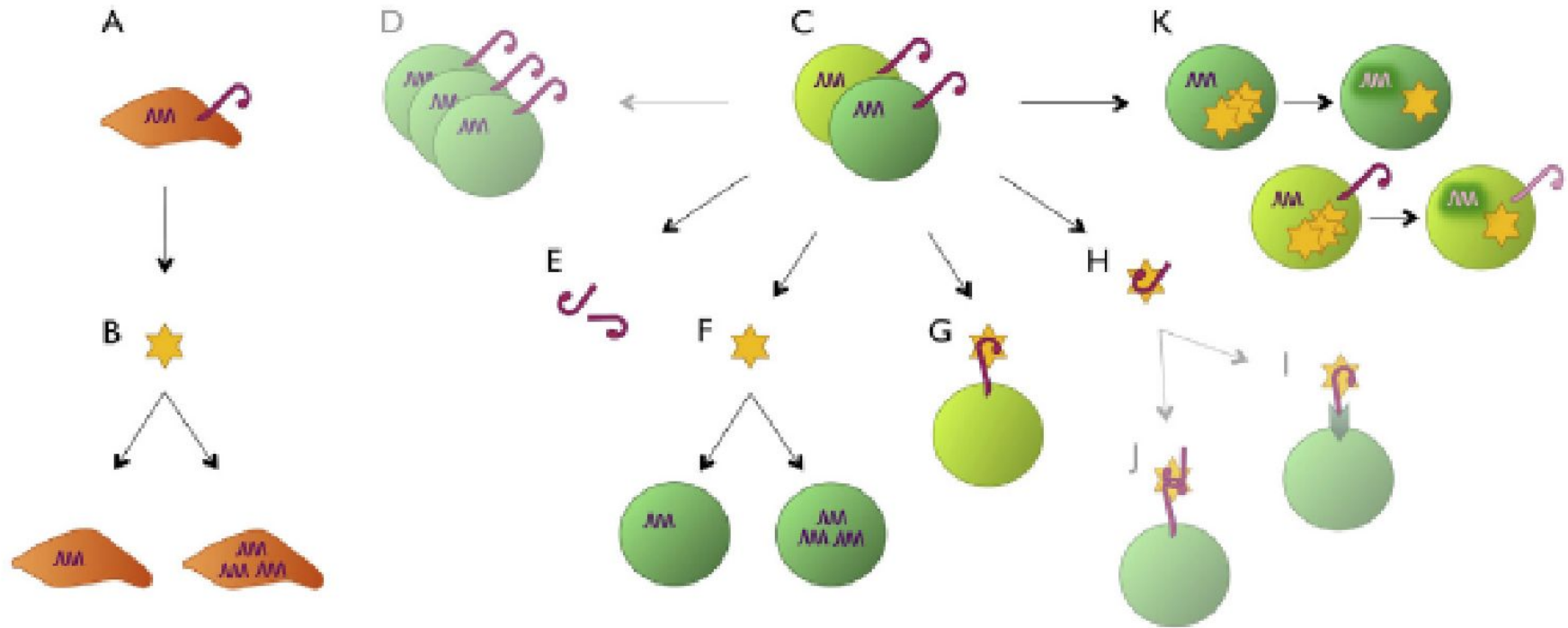
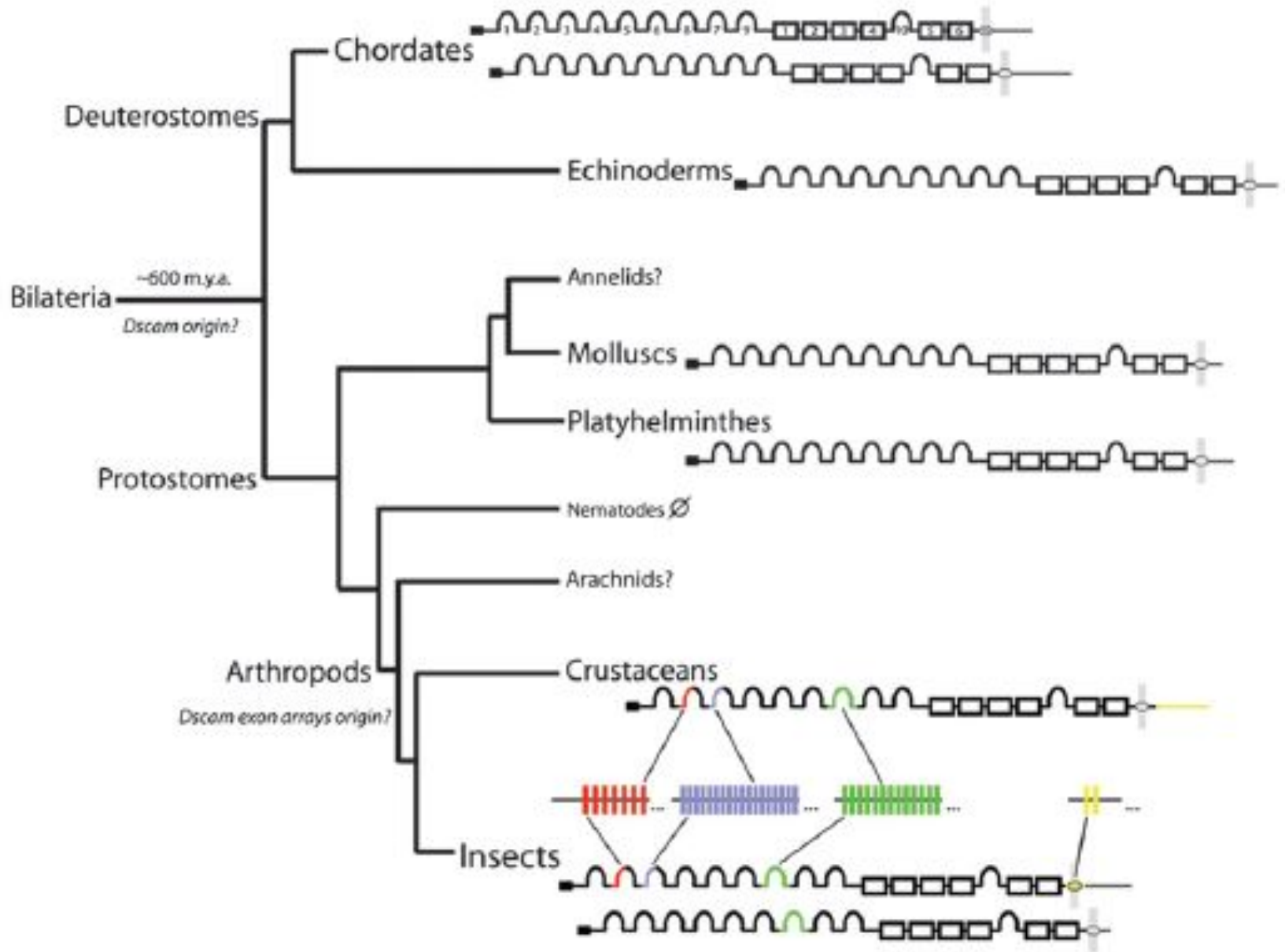
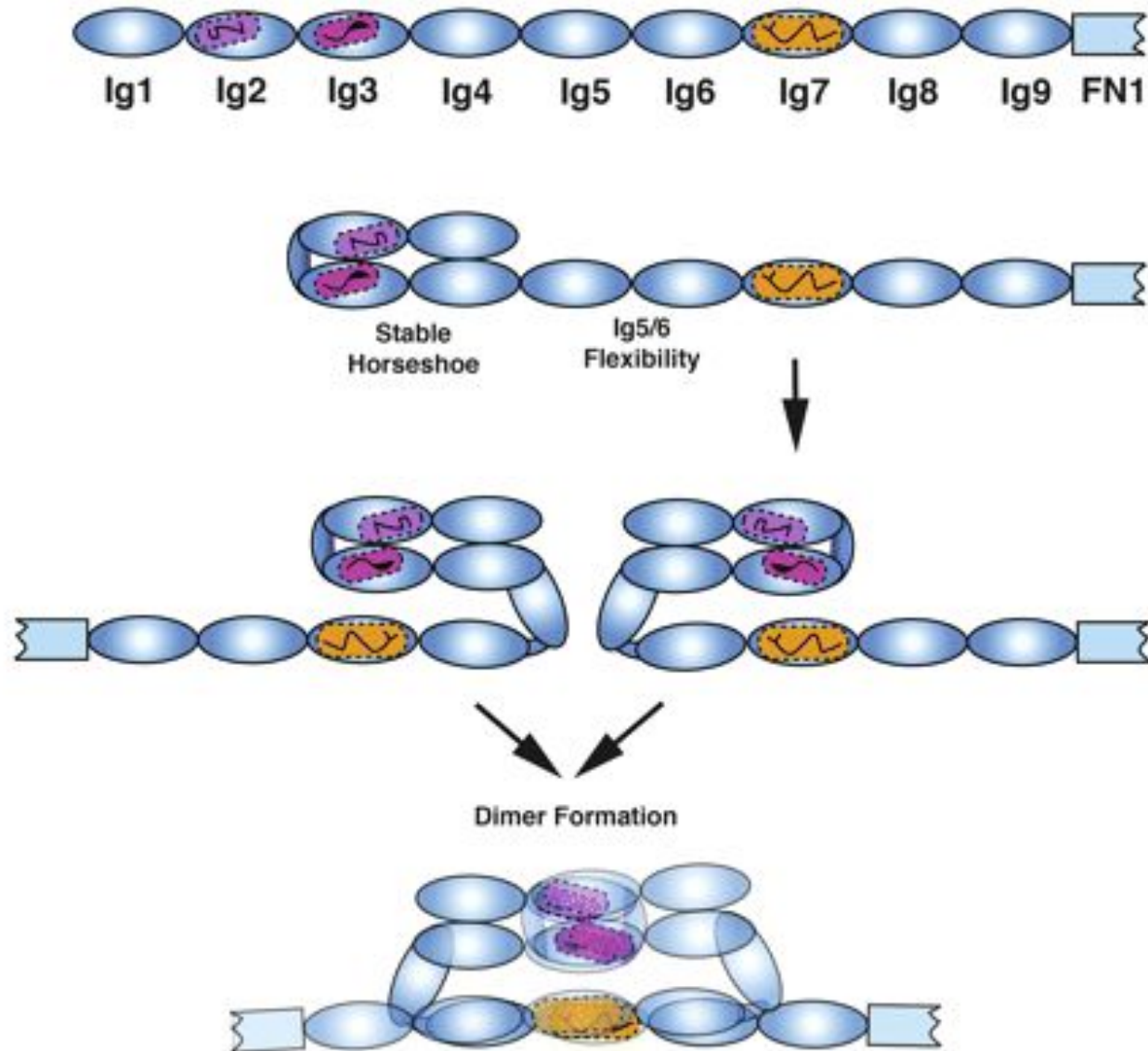


Fig. 2. Scheme of some experimentally supported (letters shown in black) and hypothesised (letters shown in grey) activities of *Dscam* in relation to pancrustacean immune tissues and responses to parasites. *Dscam* mRNA is shown as a squiggle inside the cells and the protein as a hook, with the first four *Dscam* Ig domains illustrated in a horse-shoe 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).

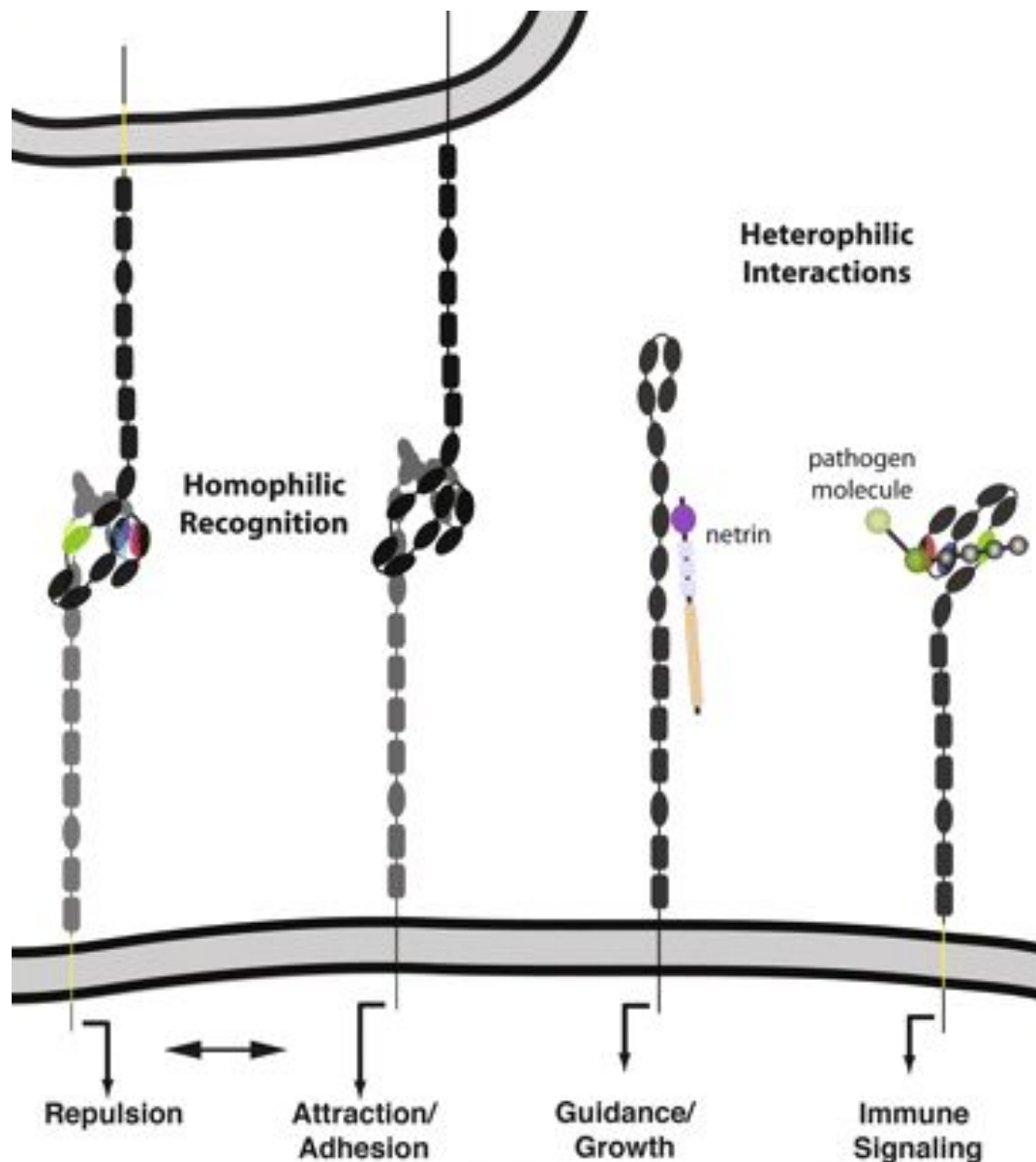
Только членистоногие имеют каскеты экзонов в гене DSCAM



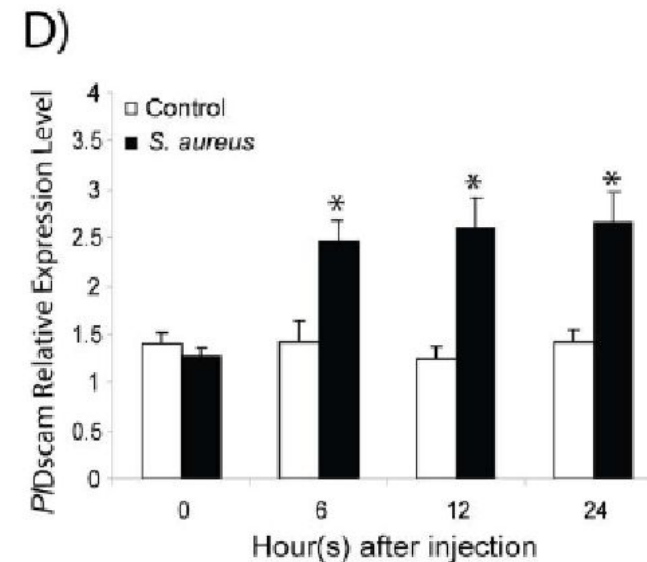
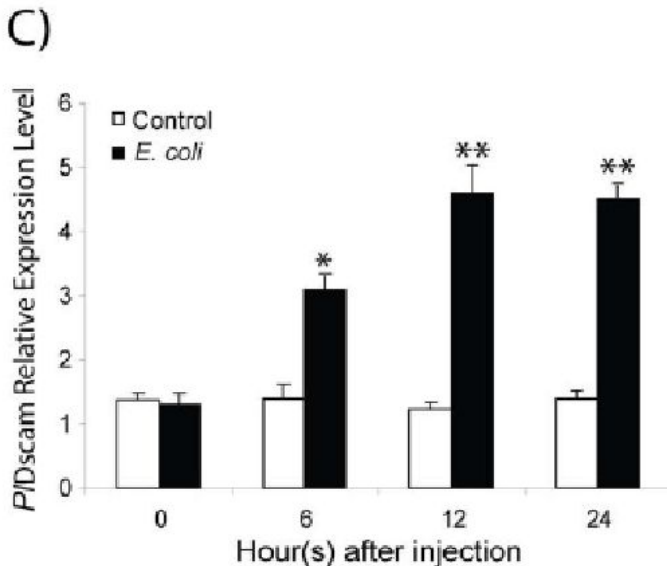
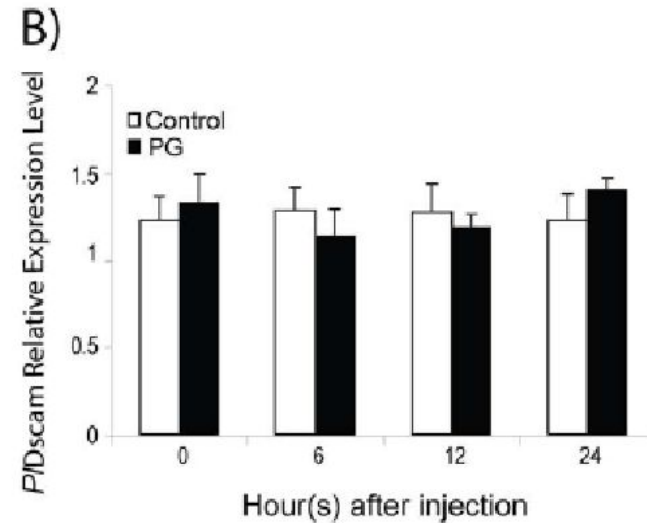
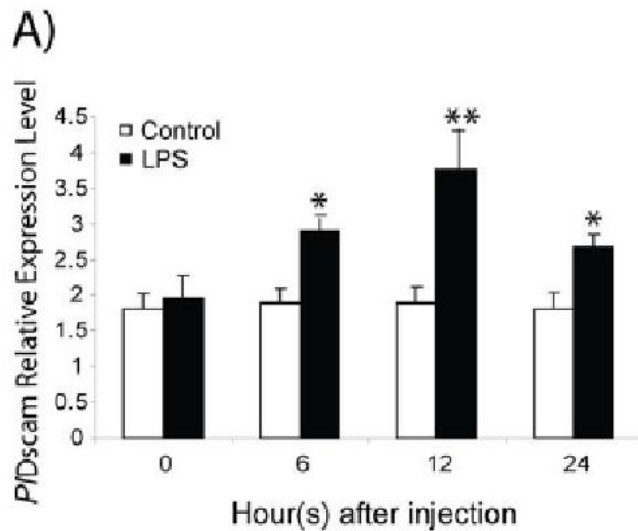
Гомофильные взаимодействия Dscam...



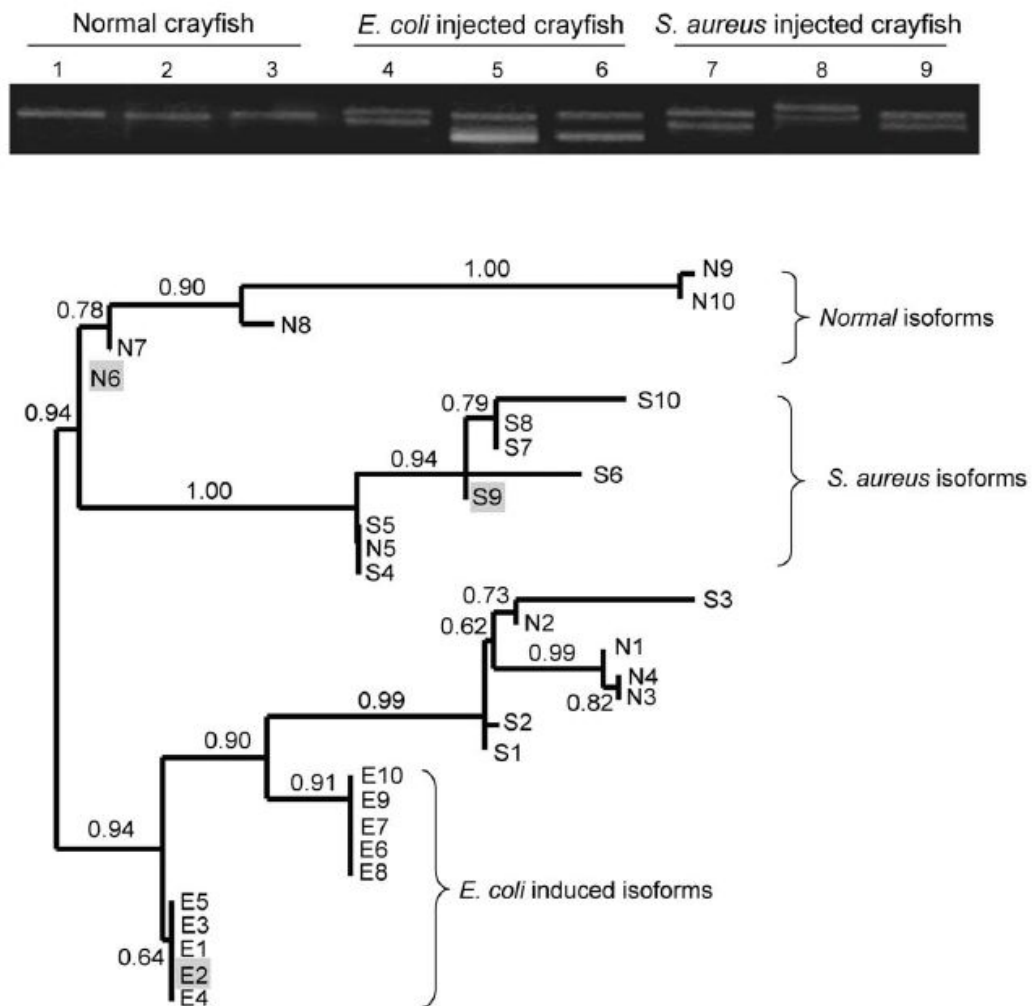
...и гетерофильные взаимодействия



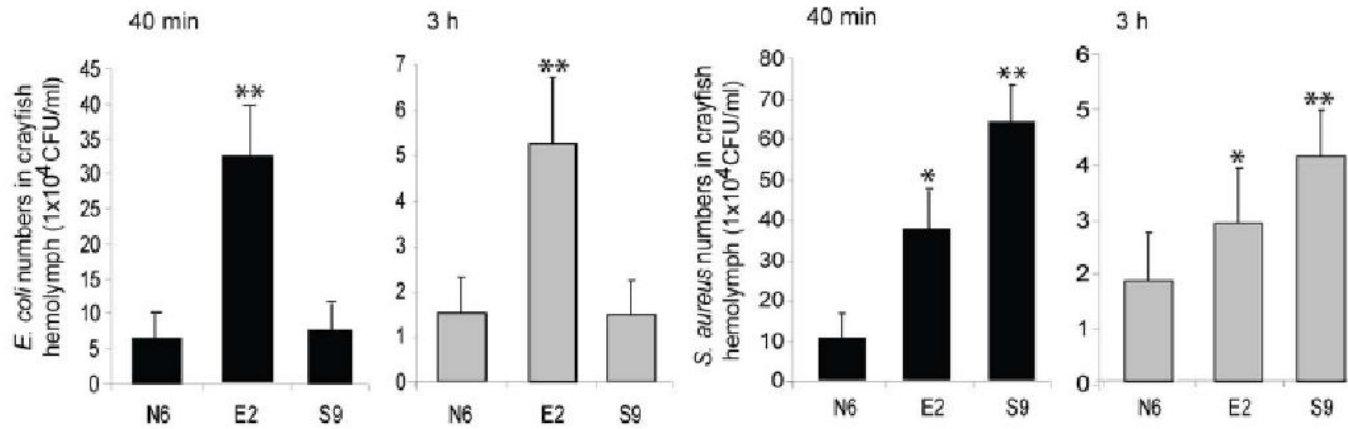
Для анофелеса и речного рака есть данные, указывающие на повышение экспрессии *Dscam* в присутствии патогенов



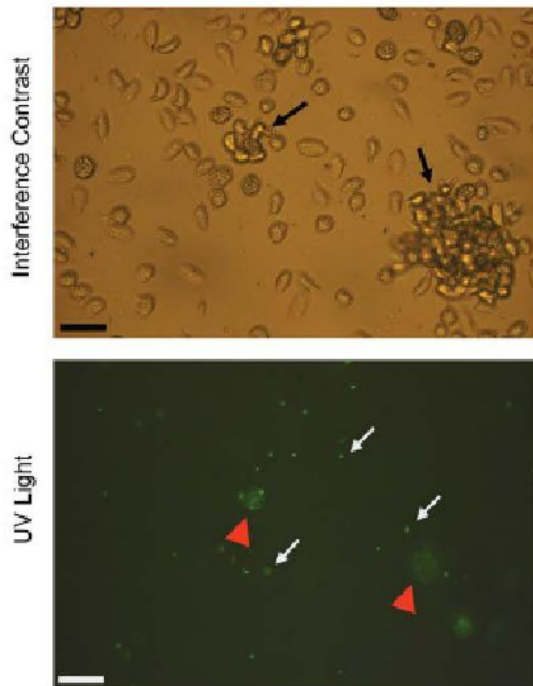
При этом после инъекции патогена в гематоцитах экспрессируются изоформы Dscam, отличающиеся от рутинных



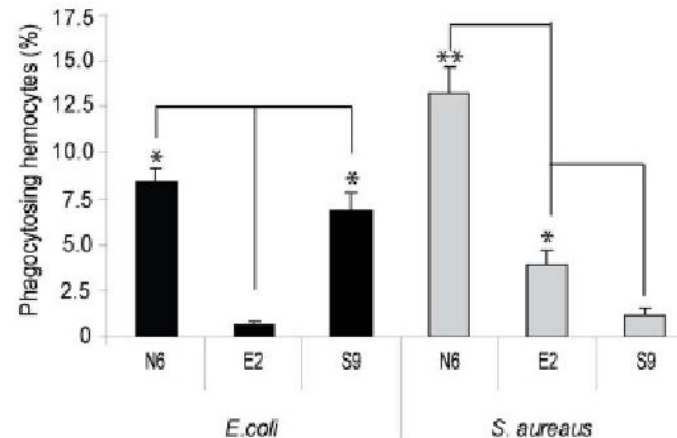
Обработанные рекомбинантными Dscam-белками бактерии подавляют фагоцитарную активность гемоцитов и дольше выживают в гемолимфе



E)



F)



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

Вольбахия защищает дрозофил от вирусов?

INFECTIVE AGENT

HOST

PROTECTION?

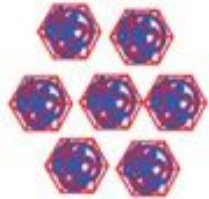
Bacteria
(*Salmonella*, *Listeria*)

Drosophila melanogaster
carrying *Wolbachia* (strain wMel)



NO

RNA viruses
(DCV, FHV, Nora)

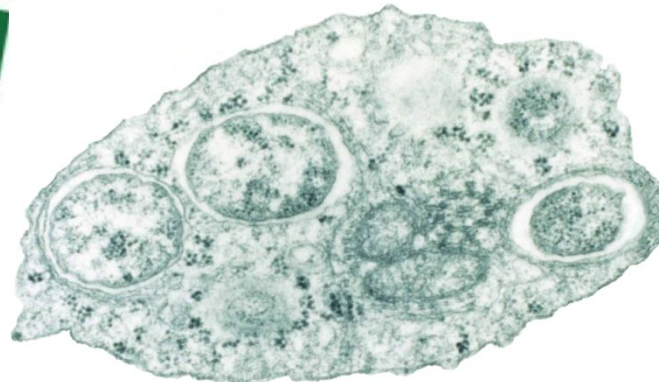
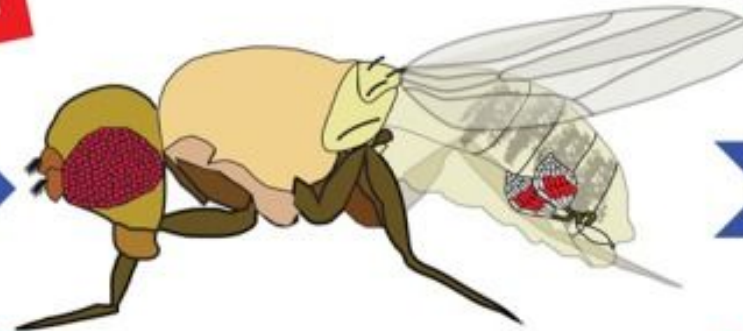


Yes

Parasitoids
(*Leptopilina*)



NO



- Следующая лекция 19 марта – об
иммунных системах других
беспозвоночных