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Expression of the Scavenger Receptor Class B type I (SR-BI) family in *Drosophila melanogaster*

LEIRE HERBOSO, ANA TALAMILLO*, CORALIA PÉREZ and ROSA BARRIO*

CIC bioGUNE, Bizkaia Technology Park, Derio, Spain

ABSTRACT In mammals, cholesterol is transformed into steroid hormones in the adrenal gland, the ovaries or the testes. The Scavenger Receptors Class B Type I (SR-BI) are membrane proteins that belong to the CD36 family and participate in the selective uptake of high density lipoprotein cholesteryl ester in the mammalian steroidogenic tissues. Fourteen members of the CD36 family have been identified in Diptera, although their expression patterns remain uncharacterized. Using *in situ* hybridization we have characterized the expression patterns of the fourteen SR-BIs in *Drosophila melanogaster*. We analyzed three different developmental larval stages prior to and during the peak of the insect steroid hormone ecdysone, which triggers the larval to pupal transition. We focused on the steroidogenic tissues, such as the prothoracic gland, the ovaries and the testes, and extended our analysis to non-steroidogenic tissues, such as the fat body, salivary glands, the gut, the gastric caeca or the central nervous system. Our results show highly regulated expression patterns, with three genes *crq, pes* and *Snmp* being upregulated in steroidogenic tissues at the onset of pupariation when steroidogenesis is crucial. This study underlines the importance of the transport of cholesterol and steroids in the process of ecdysone synthesis.

KEY WORDS: *Drosophila, scavenger receptor, CD36, ecdysone, steroidogenesis, expression pattern*

Steroid hormones regulate a wide variety of developmental and physiological processes in higher organisms. In mammals, these hormones are synthesized from cholesterol in steroidogenic tissues, such as the adrenal gland, ovaries or testes. In these tissues, as well as in the liver, the Scavenger Receptor Class B type I (SR-BI) is one of the receptors that participate in the selective uptake of cholesterol, mainly in the form of High Density Lipoprotein cholesteryl ester (HDL-CE) (Acton *et al.*, 1996). SR-BI together with CD36, CLA1 and LIMPII belong to the Cluster of Differentiation 36 (CD36) family, that contain two-transmembrane domains and they are often referred to as fatty acid transporters or Scavenger Receptors.

The surface of the steroidogenic cells are endowed with an intricate microvillar system, specialized on lipoprotein trapping (Azhar *et al.*, 1988; Reaven *et al.*, 1984, 1986, 1988, 1989, 1990). This constitutes the microvillar compartment, and the space created between adjacent microvilli forms the microvillar channels (Reaven *et al.*, 1988, 1989, 1990). SR-BI is able by itself to promote the formation of microvillar channels when over-expressed in insect Sf9 cells (Reaven *et al.*, 2001). These channels can trap HDL particles and initiate massive selective uptake of cholesteryl esters. Furthermore, SR-BI is necessary for the formation of the microvillar channels of the adrenal gland, which are disorganized in Srb1$^{-/-}$ mice (Williams *et al.*, 2002).

In addition, SR-BI can cause changes in the cholesterol and/or phospholipids composition of microvillar membranes and it is thought to provoke the formation of specific lipid rafts necessary for microvillar channel formation (Connelly *et al.*, 2001; de La Llera-Moya *et al.*, 1999, 2001; Kellner-Weibel *et al.*, 2000). SR-BI also affects the flux of free cholesterol and properties of the plasma membrane. In addition, the expression of SR-BI and the number and complexity of the microvillar compartment increases in adrenal glands of rats treated with Adeno-Corticotropic Hormone (Azhar *et al.*, 2002), illustrating the relationship between SR-BI expression and the structural configuration of the surface of steroidogenic cells.

Twelve to fourteen CD36 homologues have been identified

Abbreviations used in this paper: 20E, 20-hydroxyecdysone; AEL, after egg laying; CNS, central nervous system; crq, croquemort; emp, epithelial membrane protein; HDL-CE, high density lipoprotein cholesteryl ester; L3, third instar larvae; ninaD, neither inactivation nor after-potential D; pes, peste; PG, prothoracic gland; PTTH, prothoracicotropic hormone; santa-maria, scavenger receptor acting in neural tissue and majority of rhodopsin is absent; SR-BI, Scavenger Receptor Class B type I.

***Address correspondence to:** Rosa Barrio or Ana Talamillo. CIC bioGUNE, Bizkaia Technology Park, 48160 Derio, Spain.
E-mail: rbarrio@cicbiogune.es ; atalamillo@cicbiogune.es

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in the dipterans *D. melanogaster, D. pseudoobscura, Anopheles gambiae* and *Aedes aegypti* (Nichols and Vogt, 2008). In *D. melanogaster*, the majority of the fourteen CD36 genes identified are uncharacterized. Some of them, such as *epithelial membrane protein (emp), croquemort (crq), neither inactivation nor afterpotential D (ninaD), scavenger receptor acting in neural tissue and majority of rhodopsin is absent (santa-maria)* and *peste (pes),* have been linked to a variety of functions, including the immune response, cell adhesion, phototransduction and autophagic cell death, among others. However, their expression patterns during postembryonic development remain mostly uncharacterized. We are particularly interested in the function of these genes in steroidogenic tissues, such as the Prothoracic Gland (PG) (Gilbert *et al.*, 2002), the ovaries and testes, where the implication of these receptors on the cholesterol intake for steroids synthesis remains to be demonstrated. To approach this question, it is necessary to investigate the expression patterns of these genes, paying special attention to the steroidogenic tissues and to one developmental window when high levels of ecdysteroids are required: the onset of pupa formation.

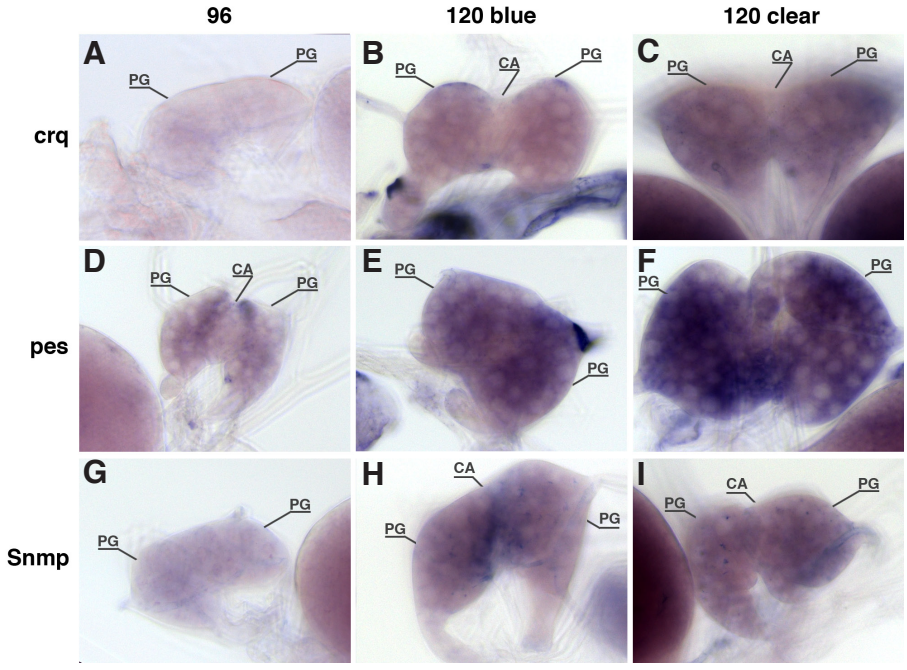

Fig. 1. Temporal regulation of the expression of SR-BIs in the ring gland. *Expression of* crq **(A-C)**, pes **(D-F)** *and* Snmp **(G-I)** *in the ring glands of 96 h AEL larvae* **(A,D,G)**, *120 h AEL blue-gut larvae* **(B,E,H)** *or 120 h AEL clear-gut larvae* **(C, F, I)**. crq, pes *and* Snmp *expression increased with time.*

Here we report the expression patterns of the fourteen CD36 *D. melanogaster* genes in various tissues in three developmental stages close to the moment of pupariation. Our results show that these genes are highly regulated temporally and spatially. Furthermore, some of them are upregulated at the end of the third instar larvae stage (L3), suggesting specific functions in steroidogenic tissues.

Results

To analyze the expression of the fourteen SR-BI genes, we performed *in situ* hybridizations in *D. melanogaster* L3 instar larvae. We analyzed three developmental stages, which we differentiated with the help of food containing bromophenol blue. At 96 h after egg laying (AEL), larvae are in the third instar and spend most of their time eating; therefore they rarely leave the food from where they were collected. At 120 h AEL, larvae abandon the food although their gut still contains the blue colorant. Later, the larvae empty their gut, synthesize high levels of ecdysone and will enter into pupariation. We selected these as 120 h AEL clear-gut larvae and corroborated that already received the ecdysone pulse after dissection according to the morphology of their salivary glands (Mitchell *et al.*, 1977). We focused our analysis in the steroidogenic tissues such as PG, ovaries and testes. We analyzed also tissues involved in metabolism, some of them involved in the conversion of ecdysone to 20-hydroxyecdysone (20E) such as the fat body, midgut and Malpighian tubules and other tissues such as the central nervous system (CNS), salivary gland and gastric caeca. As controls, we did *in situ* hybridizations using the sense RNA for each gene (data not shown).

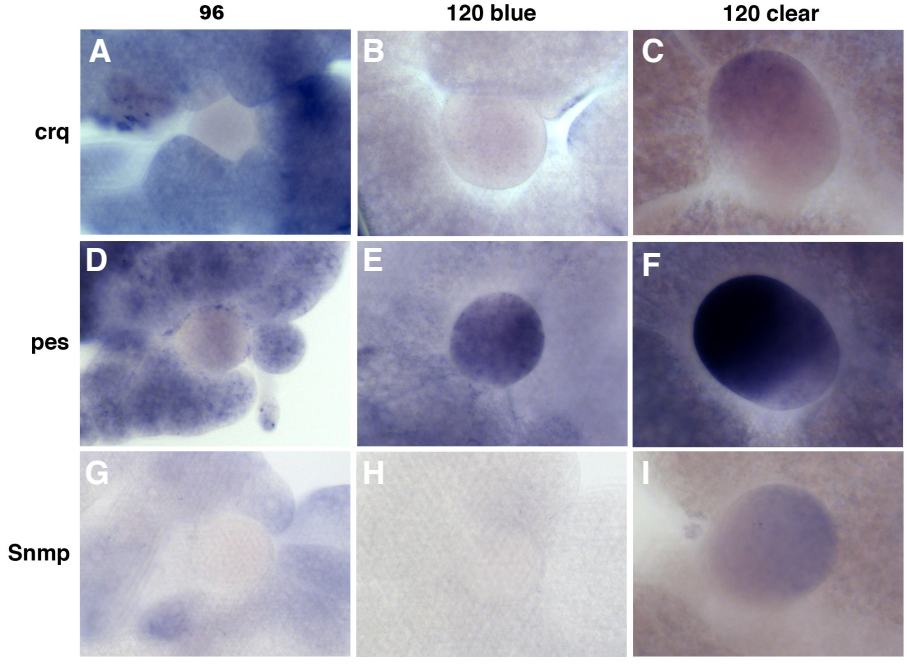

Fig. 2. Temporal regulation of the expression of the SR-BIs in the ovaries. *Expression* crq **(A-C)**, pes **(D-F)** *and* Snmp **(G-I)** *in the ovaries of 96 h AEL larvae* **(A,D,G)**, *120 h AEL blue-gut larvae* **(B,E,H)** *and 120 h AEL clear-gut larvae* **(C,F,I)**. *In all cases, the expression is upregulated in 120 h clear-gut larvae, being restricted to either the anterior or posterior parts of these ovaries.*

TABLE 1

TEMPLATES USED TO SYNTHESIZE THE RNA PROBES

Gene	Template
CG10345	GH08773
CG1887	IP12356
CG2736	GH15894
CG31741	PCR product
CG3829	GH19047
CG40006	RE68569
CG7227	GH07959
CG7422	IP10059
crq	RE02070
emp	RE08130
ninaD	RE41741
pes	RE21078
santa-maria	GH23019
Snmp	IP13851

Expression of SR-BIs in steroidogenic tissues

Ring gland

The ring gland is a specialized structure in Diptera that contains both ecdysone secreting (PG) and juvenile hormone secreting (corpus allatum) cells, as well as the corpus cardiacum. It is connected to certain neurons in the CNS, constituting the hormonal regulatory organs equivalent to the hypothalamic-pituitary-adrenal axis in vertebrales. We detected expression in the ring gland of three SR-BIs: *crq, pes* and *Snmp* (Fig. 1; Table 2). These are upregulated during development, with *pes* being the only one present at 96 h AEL. *crq* and *Snmp* seem to be restricted to the PG, while a subtle expression of *pes* can also be detected in the corpus allatum.

Ovaries and Testes

The gonads are part of the reproductive organs and contain the gametes. Fig. 2 and Fig. 3 show the patterns of all the SR-BIs expressed in ovaries or testes. *crq, pes* and *Snmp* are the only SR-BIs expressed in ovaries (Fig. 2; Table 2). *crq* and *Snmp* are

TABLE 2

EXPRESSION PROFILE OF THE FOURTEEN *D. MELANOGASTER* SR-BI GENES AT THREE DEVELOPMENTAL TIMES IN THE INDICATED TISSUES

				Gonads		Alimentary tract							
		RG	FB	Ovary	Testis	Gastric caeca	Midgut	Hindgut	Malpighian tubules	Garland cells	Anterior spiracles	SG	CNS
CG10345	120 clear	-	-	-	+++	-	-	-	-	+[1]	-	-	-
	120 blue	-	-	-	++	-	-	-	-	-[1]	++	-[2]	-
	96	-	-	-	+	-	-	-	-	-[1]	++	-	-
CG1887	120 clear	-	+[2]	-	++	-	-	-	-	++	-	+[2]	++
	120 blue	-	++[2]	-	++	-	-[2]	-	-	+	++	+[2]	+++
	96	-	-	-	+	-	-	-	-	++	+++	-[2]	+
CG2736	120 clear	-	-	-	-	-	-	-	-	++	-	-	-
	120 blue	-	++[2]	-	-	-	-	-	-	++	-	-	-
	96	-	+++[2]	-	-	-	-	-	-	++	-	-	-
CG31741	120 clear	-	-	-	-	-	-	-	-	++	-	-	-
	120 blue	-	-	-	-	-	+[2]	-	-	++	-	+	-
	96	-	-	-	-	-	-	-	-	++	-	-	-
CG3829	120 clear	-	+++[2]	-	+++	+++[2]	+++[2]	-	++	++	-	+[2]	-
	120 blue	-	++[2]	-	++	+[2]	+[2]	-	-	-	-	-	-
	96	-	+	-	+	-	-	-	-	-	-	-	-
CG40006	120 clear	-	-	-	-	-	-	-	-	+	-	-	-
	120 blue	-	-	-	-[1]	-	-	-	-	+	-	+	-
	96	-	-	-	-	-	-	-	-	-	-	-	-
CG7227	120 clear	-	-	-	+++	-[2]	+[2]	-	-	+[1]	-	-[2]	-
	120 blue	-	+	-	++	-[2]	-[2,1]	-	-	+	-	+[2]	-
	96	-	-	-	+	-[2]	-[2]	-	-	+	-	-	-
CG7422	120 clear	-	-	-	+	-	-	-	-	-[1]	-	-[2]	-
	120 blue	-	-	-	++	-[2]	-[2]	-	-	-[1]	-	-[2]	-
	96	-	-	-	++	-	-[2]	-	-	-[1]	++	-	-
crq	120 clear	++	-	+	++	++	+++	-	-	++	-	+[2]	+
	120 blue	+	-	-	+	+	++	-	-	+	-	-	-
	96	-	-	-	+	-	+	-	-	+	-	+	-
emp	120 clear	-	-	-	+++	-	-	-	-	-	+++	-	-
	120 blue	-	-	-	+++	-	-	-	-	-	+++	-	-
	96	-	-	-	+++	-	-	-	-	-	+++	-	-
ninaD	120 clear	-	-	-	-	-	-	-	-	++	-	-	-
	120 blue	-	+	-	-	-	+++	-	+[3]	++	-	-	-
	96	-	++[3]	-	-	-	+++	-	+[3]	++	-	-	-
pes	120 clear	+++	+++	+++	+++	+++	++	++	++	++	+++	+++[2]	+++
	120 blue	++	++	+	+++	+++	+++[2]	++	++	++	++	+++[2]	++
	96	+	+	-	+++	+++	+++[2]	++	++	++	+	+++	+
santa-maria	120 clear	-	+++	-	-	-	++[2]	-	-	++	-	-	+++
	120 blue	-	-	-	-	-	-[2]	-	-	-	-	-	++
	96	-	-	-	-	-	-[2]	-	-	-	-	-	+
Snmp	120 clear	++	-	+	+++	-	-	-	-	+	-	+[2]	+
	120 blue	++	+[3]	-	++	-	-	-	-	+	-	++[2]	-
	96	-	-	-	++	-	-	-	-	+	-	-[2]	-

-: non- detectable expression; +: weak expression; ++: moderate expression; +++: strong expression; 1: non conclusive result; 2 accumulation of RNA inside the nuclei; 3: expression in scattered cells.

only expressed in 120 h clear-gut larvae, while the expression of *pes* became apparent in 120 h blue-gut larvae and increased close to the moment of pupariation. The spatial expression of the three SR-BIs is restricted to one pole of the ovary, especially evident for *pes*.

Nine of the SR-BIs are expressed in the testes: *CG10345*, *CG1887*, *CG3829*, *CG7227*, *CG7422*, *crq, emp, pes*, and *Snmp* (Fig. 3; Table 2). Some genes, like *CG7227*, *pes,* or *Snmp* are expressed throughout the testis, while some others are restricted to the anterior (*crq*), the posterior (*CG10345*) or the central parts (*CG1887*, *CG3829* or *CG7422*). *emp* extended its expression from the posterior part to the whole testis. With respect to the temporal regulation, the patterns are highly variable, with expression being constant for *pes*, upregulated for *CG10345*, *CG1887*, *CG3829*, *CG7227*, *crq* and *Snmp* or downregulated for *CG7422*.

Expression of SR-BIs in other tissues

Fat body

The fat body is the main energy storage tissue of the organism and is also implicated in the immune response by the production of antimicrobial peptides and in the conversion of ecdysone to 20E. Ecdysone 20-monooxygenase, the enzyme that hidroxylates ecdysone to 20E, is also present in other tissues peripheral to the PG such as the Malpighian tubules and the midgut (Petryk *et al*., 2003). Fig. 4 shows the eight SR-BIs expressed in the fat body: *CG1887*, *CG2736*, *CG3829*, *CG7227*, *ninaD*, *pes, santa-maria*, and *Snmp* (Table 2). *ninaD* and *Snmp* show a heterogeneous pattern at 96 h AEL and 120 h blue-gut, respectively, some cells showing higher expression than others (Fig. 4 M,W). The expression of some of these genes follows a temporal regulation. *CG3829* and *pes* are upregulated throughout development, while *CG2736* and *ninaD* are downregulated. *CG1887* is upregulated in 120 h blue-gut larvae and downregulated in 120 h clear-gut larvae. *santa-maria* is only expressed in 120 h clear-gut larvae and *CG7227* in 120 h blue-gut larvae.

Alimentary tract

The alimentary tract includes the proventriculus, the gastric caeca, the garland cells, the midgut, the hindgut and the Malpighian tubules. The gut and the gastric caeca are the principal organs involved in the secretion of digestive enzymes and the digestion and absorption of the food. The gut plays also an important role in the immune response. The Malpighian tubules, joined to the alimentary tract at the junction between the midgut

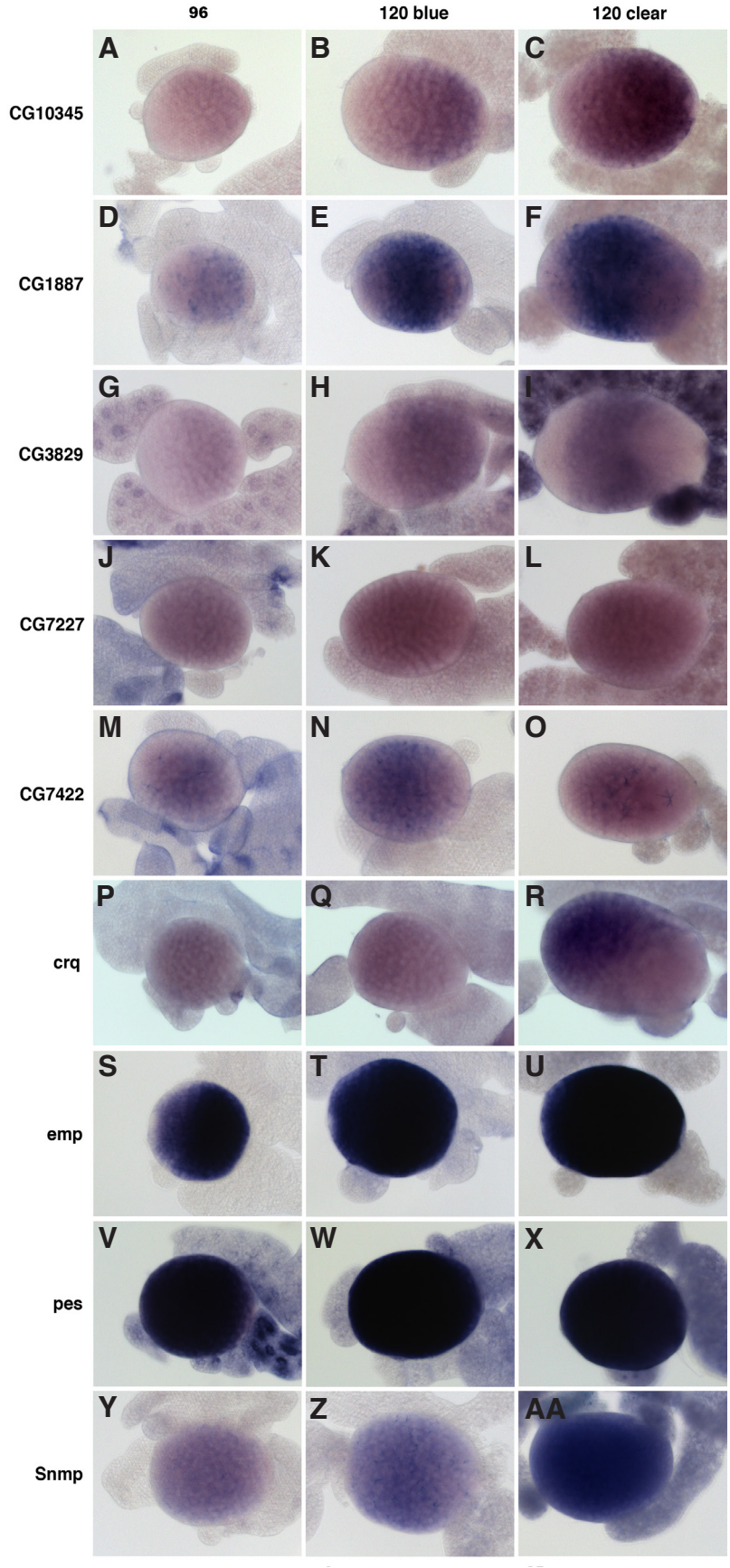

Fig. 3. Temporal regulation of the expression of the SR-BIs in the testes. *Expression of CG10345* **(A-C)**, *CG1887* **(D-F)**, *CG3829* **(G-I)**, *CG7227* **(J-L)**, *CG7422* **(M-O)**, crq **(P-R)**, emp **(S-U)**, pes **(V-X)** *and* Snmp **(Y-AA)** *in the testes of 96 h AEL larvae* **(A,D,G,J,M,P,S,V,Y)**, *120 h AEL blue-gut larvae* **(B,E,H,K,N,Q,T,W,Z)** *or 120 h AEL clear-gut larvae* **(C,F,I,L,O,R,U,X, AA)**. *Anterior is to the left.*

and hindgut, perform excretory and osmoregulatory functions. In *Drosophila* the contribution of midgut and Malpighian tubules in steroidogenesis may be less significant than the fat body. Little is known about the function of the garland cells, although they are considered to be athrocytes, cells that uptake material from the hemolymph and are highly active in clathrin-mediated endocytosis. Twelve out of the fourteen SR-BIs are expressed in at least one of the components of the alimentary tract, with *pes* being the most ubiquitous (Table 2).

CG3829, *crq* and *pes* are expressed in the gastric caeca (Table 2). *CG3829* and *crq* are upregulated during development, while *pes* expression is constant. *crq* shows a heterogeneous pattern, with the level of expression differing among cells (Fig. 5 A-C).

Seven of the SR-BIs are expressed in the midgut: *CG31741*, *CG3829*, *CG7227*, *crq, ninaD*, *pes* and *santa-maria* (Table 2). The expression of *CG3829*, *CG7227*, *crq* and *santa-maria* increased during development, while the expression of *ninaD* and *pes* decreased. At 96 h AEL *ninaD* is expressed throughout the midgut with the exception of the most anterior portion. This pattern is broader in blue-gut larvae at 120 h, with the limit of the expression being shifted anteriorly, and is downregulated in the entire gut just before pupariation (Table 2 and Fig. 5 G-I). By contrast, *pes* is the only SR-BI expressed in the hindgut, with no evidence of temporal regulation.

CG3829, *ninaD* and *pes* are expressed in the Malpighian tubules (Table 2). *pes* expression is constant during development while *CG3829* is upregulated and *ninaD* is downregulated with time. The expression of *ninaD* is restricted to a few cells localized in the part in closer proximity to the gut (Fig. 5 D-F).

CG10345, *CG1887*, *CG2736*, *CG31741*, *CG3829*, *CG40006*, *CG7227*, *crq, ninaD*, *pes, santa-maria* and *Snmp* are expressed in the garland cells (Table 2). *CG10345*, *CG3829*, *CG40006*, *crq* and *santa-maria* expression is upregulated during development, while *CG2736*, *CG31741*, *CG7227*, *ninaD*, *pes* and *Snmp* expression is not temporally regulated.

Anterior spiracles

The spiracles are the external tracheal apertures, which contain spiracular glands that produce lipids secretions. These coat the surface of the spiracular plate and branched hairs (Keilin, 1944; Keilin *et al.*, 1935; Rizki, 1956). *CG10345*, *CG1887*, *CG7422*, *emp* and *pes* are expressed in this organ (Table 2). *CG10345*, *CG1887* and *CG7422* are downregulated throughout development, while *pes* is upregulated and *emp* remains constant. The expression patterns of these genes show spatial restrictions. *CG10345*, *CG1887*, *CG7422* and *emp* are expressed at some developmental times in the

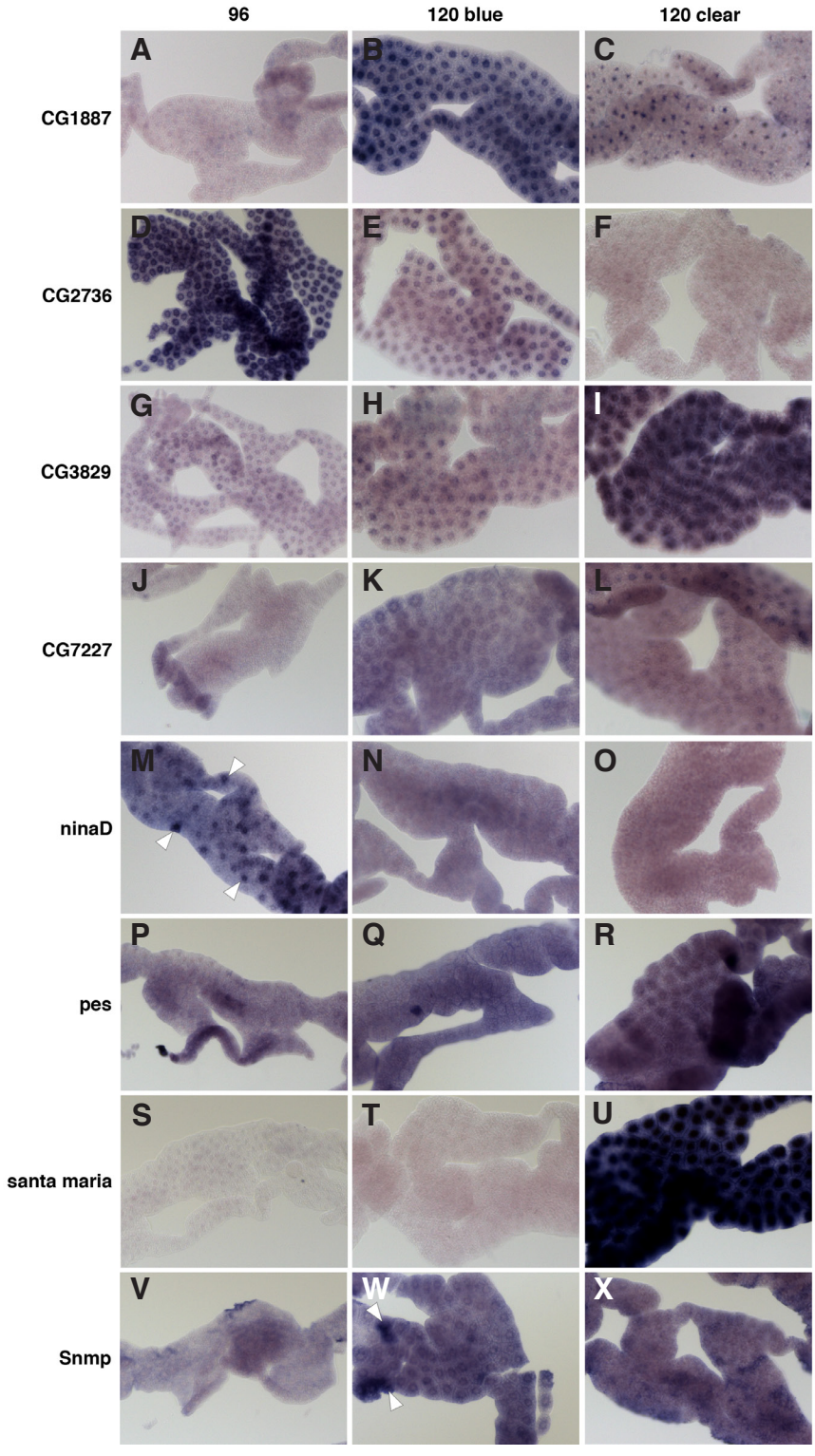

Fig. 4. Temporal regulation of the expression of the SR-BIs in the fat body. *Expression of* CG1887 **(A-C)**, CG2736 **(D-F)**, CG3829 **(G-I)**, CG7227 **(J-L)**, ninaD **(M-O)**, pes **(P-R)**, santa maria **(S-U)** *and* Snmp *CG31741* **(V-X)** *in the fat body of 96 h AEL larvae* **(A,D,G,J,M,P,S,V)**, *120 h AEL blue-gut larvae* **(B,E,H,K,N,Q,T,W)** *or 120 h AEL clear-gut larvae* **(C,F,I,L,O,R,U,X)**. *Arrowheads in* **(M,W)** *indicate cells with higher expression than the others.*

cells of the gland closer to the spiracular plate, while *pes* is expressed throughout the gland (Fig. 5 J-O).

Salivary gland

The salivary glands are accessory organs for digestion. When the larvae are going to enter into pupariation, salivary glands secrete the glue proteins, which allow the larvae to stick to a surface until the adult emerges from the pupal case. Eight SR-BIs are expressed in the salivary gland: *CG1887*, *CG31741*, *CG3829*, *CG40006*, *CG7227*, *crq*, *pes* and *Snmp*. Among those, *pes* is the only one highly expressed throughout the three developmental times analyzed, while the others are temporally regulated (Table 2).

Central nervous system

CG1887, *crq*, *pes, santa-maria,* and *Snmp* are expressed in the CNS (Table 2). *crq*, *pes*, *santa-maria* and *Snmp* are upregulated during development, while *CG1887* shows higher expression in 120 h blue-gut larvae. These genes show spatially restricted patterns. *CG1887*, *crq* and *pes* are expressed in the brain hemispheres and the more anterior part of the ventral ganglion. *Snmp* expression is restricted to the brain hemispheres. Expression of *santa-maria* appeared in the brain hemispheres of 96 h larvae, but in 120 h clear-gut larvae the expression became ubiquitous (Fig. 5 P-U).

Discussion

The CD36/SR-BI gene family in mammals is composed by four members, CD36, CLA1, LIMPII and SR-BI, which show a broad range of functions and widespread expression patterns; CD36 is expressed in blood cells, adipocytes,

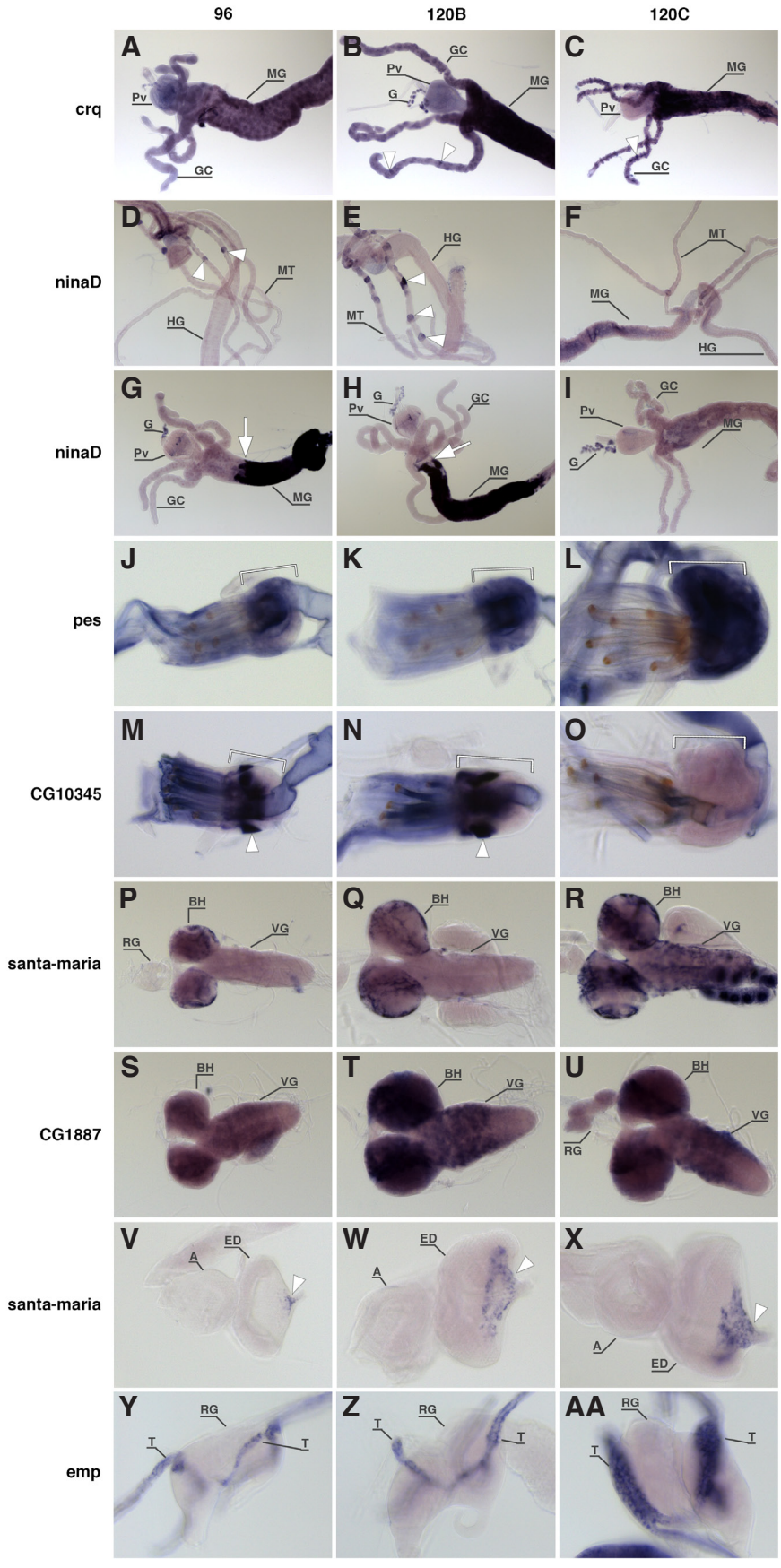

Fig. 5. Temporal regulation of the SR-BIs in non esteroidogenic tissues. *Non-homogeneous expression of* crq **(A-C)** *in gastric caeca* **(GC)**, *arroheads indicate cells whit a higher level of expression, (Pv: proventiculus, MG: midgut, G: garland cells). Restricted expression of* ninaD **(D-F)** *to a few cells (arrowheads) of the Malphigina tubules* **(MT)** *and* **(G-I)** *shows the particular expression pattern in the MG, arrows indicate the point where the expression stars. Expression in the anterior spiracles of* pes **(J-L)** *in the whole spiracular gland (indicated by brace) and CG10345* **(M-O)** *in specific cells of the gland (arrowheads). Expression of* santa-maria **(P-R)** *and CG1887* **(S-U)** *in the CNS (RG: ring-gland, BH: brain hemispheres, VG: ventral ganglio). Expression of* santa-maria **(V-X)** *in eye-antennal imaginal disc, arrowheads indicate the photoreceptor cluster (A: antennal segment, ED: eye disc). Expression of* emp **(Y-AA)** *in tracheas (T).* **(A,D,G,J,M,P,S,V,Y)** *correspond to 96 h AEL larvae,* **(B,E,H,K,N,Q,T,W,Z)** *to 120 h AEL blue-gut larvae and* **(C,F,I,L,O,R,U,X,AA)** *to 120 h AEL clear-gut larvae. Anterior is to the left.*

endothelial cells and retinal and mammary epithelia. There, CD36 is implicated in cell adhesion, phagocytosis of apoptotic cells and signal transduction, as well as in transport and absorption of lipoproteins and fatty acids (Abumrad *et al.*, 1993; Greenwalt *et al.*, 1992; Oquendo *et al.*, 1989; Ryeom *et al.*, 1996); CLA1 is expressed in the adrenal gland, liver, testes, monocytes and macrophages. It binds to apoptotic thymocytes and acts as a lipoprotein receptor (Calvo and Vega, 1993; Murao *et al.*, 1997; Pussinen *et al.*, 2000); LIMPII localizes in lysosomal membranes and it is implicated in cell adhesion (Vega *et al.*, 1991); SR-BI is located in fat, lung, liver, ovary and adrenal gland, being also a lipoprotein receptor (Acton *et al.*, 1994; Rigotti *et al.*, 1995).

Multiple orthologues of this family have been identified in invertebrates. In the case of the nematode *C. elegans*, six CD36-like proteins have been identified, which are implicated in the engulfment of apoptotic cell. The expression of this proteins covers many tissues, such as the intestine, the hypodermal cells, and the head and tail of L1 larvae (Hoque and Chalfie, 2001). In insects, twelve to fourteen CD36 genes were identified in dipteran and coleopteran, while eight in hymenopteran. However, little information is available about the SR-BIs from other insect species outside *D. melanogaster* (Nichols and Vogt, 2008). The existence of fourteen homologues of this family could indicate a diversification of functions in arthropods (Nichols and Vogt, 2008). The physiological functions of the *D. melanogaster* members of this family are largely uncharacterized, with the exception of *crq*, *ninaD*, *emp*, *santa-maria* and *Snmp*, which show roles in immunity, absorption of carotenoid or pheromone recognition, among others. Our results suggest a role for some of these genes in steroidogenic tissues. In addition, the expression of some of them in the alimentary tract and in the fat body would suggest a function in the lipid and energy homeostasis of the organism.

In the scope of our study, where we focused our attention on three developmental times in a variety of larval tissues, we could observe highly dynamic and, in some cases, restrictive patterns of expression. Some genes show a broad expression in most of the tissues analyzed, such as *pes*, while others are more restricted, such as *CG40006* or *CG2736* (Table 2). It is also remarkable the temporal regulation that some of these genes undergo in a relatively short period of time. For instance, *santa-maria* shows a strong upregulation in the fat body in clear-gut *versus* blue-gut larvae (Fig. 4 T,U), while *CG2736* is downregulated in the same tissue (Fig. 4 D,E). *ninaD* is also visibly downregulated in the midgut during the transition from blue to clear-gut (Fig. 5 H,I). Another example of specificity is the expression of *emp* in the tracheae (Fig. 5 Y-AA).

In mammals, SR-BI is essential for the formation of microvillar channels and HDL-CE uptake in adrenal gland (Reaven *et al.*, 2001; Williams *et al.*, 2002). We report here the expression of the *D. melanogaster* SR-BIs homologues in tissues in which they could be involved in the capture of cholesterol or other lipids. A previous report showed that *ninaD* is implicated in the absorption of dietary carotenoids, which are lipid soluble tetraterpenoids (Voolstra *et al.*, 2006). Carotenoids are stored in adipose tissues and, in pupal stages, NinaD redistributes the carotenoids from adipose tissue to the developing eyes (Kiefer *et al.*, 2002). The expression of *ninaD* was also described in other larval tissues, such as the CNS, the fat body, the midgut, the hindgut and the Malpighian tubules (Voolstra *et al.*, 2006; Yang and O'Tousa, 2007). Consistent with the literature, our study corroborates the expression of *ninaD* in the fat body, the midgut and the Malpighian tubules, and reveals its expression in the garland cells. In addition, we also show that the expression of *ninaD* in the midgut is temporally regulated (Fig. 5G-I). This highly regulated pattern of expression might indicate a role for *ninaD* in the uptake of carotenoids or other lipids in the gut prior to pupariation. Another homologue, *santa-maria* has also been involved in the biosynthesis of rhodopsin, acting in neurons and glia cells, outside the retina (Wang *et al.*, 2007). Recently, a new role for retinoid biosynthesis in regulating the expression of the neuropeptide prothoracicotropic hormone (PTTH) and delaying development in response to tissue damage has been described (Halme *et al.,* 2010). Interestingly, this study suggests that *santa-maria* is partially involved in the delay of the *Ptth* gene upregulation and, therefore, in the delay of pupariation after damage to imaginal discs. We have observed *santa-maria* expression in the photoreceptor cluster of the eye-antenna imaginal discs, which is upregulated at the stages prior to pupariation. Our results are in concordance with its described function and support the role of these receptors in the process of pupariation (Fig. 5 V-X). *santa-maria*, as well as *CG3829* and *emp,* is also involved in autophagic cell death of the salivary gland during pupariation (Gorski *et al.*, 2003).

Some of the other SR-BIs also act as phagocytic receptors for apoptotic cells, bacteria and virus, playing important roles in the immune response. For instance, *crq* is expressed in *D. melanogaster* haemocytes/macrophages and it plays a specific role in the recognition and phagocytosis of apoptotic cells and *Staphylococcus aureus* (Franc *et al.,* 1996, 1999; Sears *et al.,* 2003; Stuart *et al.,* 2005). Its expression is also upregulated after a viral infection (Go *et al.*, 2006). Our results show that *crq* is upregulated in the gastric caeca, midgut and garland cells, with these tissues being the primary barrier against the ingested microbes and inhaled fungal spores. *pes* is required for the uptake of *Mycobacterium smegmatis* and *Listeria monocytogenes* by macrophages (Philips *et al.*, 2005), and its expression in midgut and salivary glands has been previously described (Cao *et al.*, 2007; Li *et al.*, 2009). *CG2736* appears to be part of the *D. melanogaster* lipid droplet subproteome (Beller *et al.*, 2006), being induced by infection with *Octosporea muscaedomesticae* (Roxstrom-Lindquist *et al.*, 2004) and upregulated in fly stocks resistant to infection with *Pseudomonas aeruginosa*, as well as *CG10345* (Ye *et al.*, 2009). In the case of *Snmp*, it is required for pheromone sensitivity in *Drosophila* and it is implicated in the reception of 11-*cis*-vaccenyl acetate (cVA), which mediates a variety of behaviours including aggregation, mate recognition and sexual behaviour. cVA is detected by a small set of olfactory neurons located in T1 trichoid sensilla on the antennae of males and females, where *Snmp* is expressed (Jin *et al.*, 2008).

SR-BIs in steroidogenic tissues

Ecdysteroid hormones, mainly ecdysone and 20E, have an important role in insect development by controlling molting and metamorphosis. In the majority of the insects, the PG is the major source of ecdysteroids during larval development, the gonads being the primary source of the hormone in the adults as the PG deteriorates. In *D. melanogaster*, ecdysone synthesis occurs primarily in the PG cells and in the ovary (Gilbert *et al.*, 2002). These tissues require high amounts of cholesterol to synthesize steroid hormones, which are responsible for insect growth and development. However, the receptors involved in cholesterol uptake in the PG remain uncharacterized. In this manuscript, we report

the identification of three SR-BI homologues expressed in the PG cells, *crq, pes* and *Snmp*, all of them being upregulated prior to pupariation, suggesting a possible function for these factors in steroidogenesis that remains to be explored.

Gonadal ecdysteroidogenesis in arthropods was first reported for ovaries of the mosquito *Aedes aegypti* (Hagedorn *et al.*, 1975) In many other insects the ovary synthesizes and accumulate ecdysteroids in the adults (Romañá *et al.*, 1995; Thompson *et al.*, 1984). In addition, in *Gryllus bimaculatus* ecdysteroids synthesis by the ovary and abdominal integument in the last instar larvae has been suggested (Gerstenlauer and Hoffmann, 1995). Larval testes are also able to synthesized ecdysteroids and release them, as demonstrated from the inner testicular sheat of *Heliothis virescens* (Loeb, 1986; Loeb *et al.*, 1982). It is interesting to note that *crq, pes* and *Snmp* are expressed in the larval ovaries and at least *crq* is expressed in adult ovaries as well (data not shown). These three genes are also expressed in testes. *pes* shows strong expression throughout the testis in all the three stages, while *crq* is restricted to the anterior part of the testis where the spermatogonia are localized and it is upregulated during development, suggesting the possibility that *crq* plays a role in the differentiation of these cells. The expression of *Snmp* in fat body and in ovary and testis is also temporally regulated, which could indicate a function for this gene in the steroidogenic tissues prior to pupariation. In contrast to the PG and the ovaries, where only these three genes are expressed at these stages, six other genes show expression in the testes, which could indicate a wider diversity of functions in this tissue.

In summary, we show here the expression of scavenger receptor homologues in *D. melanogaster* in a variety of tissues during the third instar larvae. Of particular interest is the regulation of the expression from feeding larvae to wandering larvae and the transition from blue-gut to clear-gut larvae. Our results show that *crq*, *pes* and *Snmp* are interesting candidates to be analyzed in the process of steroidogenesis during the larval to pupal transition.

Materials and Methods

Drosophila melanogaster larvae collection

Vallecas was used as wild type strain. Flies were let to lay eggs for 8 h at 25°C on tubes with food containing bromophenol blue at 0.05% (w/v) (Maroni and Stamey, 1983). Larvae were collected at three developmental moments: 96 h AEL, 120 h AEL with blue dye remaining in the gut and 120 h AEL larvae that already cleared their gut. The majority of larvae with dark blue gut will puparíate in 12-24 h; the larvae with partially cleared gut will puparíate in 5-12 h, and most of the clear gut larvae will puparíate in 1-6 h (Andres and Thummel, 1994).

RNA probe preparation and in situ *hybridization*

ESTs from the Berkeley *Drosophila* Genome Project cDNA collections or PCR product were used as templates for the synthesis of the RNA probes (Table 1). In the case of CG31741, genomic DNA was amplified using the primers CG31741–179Fw (5'–TTTACGACAACACCTTTGGCTGGTT–3') and CG31741–709Rev (5'–TGCCGAGAGTGGGCTCCATTATCAC–3'). The product of amplification was cloned into the TOPO® vector (Invitrogen).

Plasmid DNA was linearized using a restriction enzyme downstream from the cloned insert (*Bam*HI or *Xho*I, Fermentas). RNA labelling was performed using the DIG RNA labelling Mix (Roche) according to the manufacture instructions, using 1μg of the linearized DNA.

The RNA probes were hybridized to larval tissues at 55°C in 50% deionized formamide (Sigma), 5x saline sodium citrate, 50 μg/ml heparin sodium salt (Sigma), 0.1% Tween 20, and 100 μg/ml of phenol extracted sonicated salmon sperm DNA (Amersham Biosciences). Samples were incubated with anti-digoxigenin antibody (1:2000; AP Fab fragments, Roche) and signal was detected using 4-Nitro blue tetrazolium chloride and 5-Bromo-4-chloro-3-indolyl-phosphate (Roche). DAPI staining was used to determine the anterior/posterior ends of the larval testis.

Microscopy

A Zeiss Axio Imager.A1 microscope, coupled to a Zeiss AxioCam HRC photo camera, and a Zeiss Axio Imager.D1, coupled to a Zeiss AxioCam HRm, were used and the AxioVision AC (release 4.8) software. Pictures were processed using the Adobe Photoshop software.

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