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Translating Available Food Into the Number of Eggs Laid by *Drosophila melanogaster*

Jun Terashima and Mary Bownes[1]

Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

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ABSTRACT

In Drosophila and other insects egg production is related to the nutrients available. Somehow the nutritional status of the environment is translated into hormonal signs that can be "read" by each individual egg chamber, influencing the decision to either develop into an egg or die. We have shown that *BR-C* is a control gene during oogenesis and that the differential expression of *BR-C* isoforms plays a key role in controlling whether the fate of the egg chamber is to develop or undergo apoptosis.

INSECT development is affected by environmental parameters such as temperature, photoperiod, and nutrition. The induction of diapause is regulated by temperature and photoperiod in *Bombyx mori* (Xu *et al.* 1995) and Drosophila (Saunders *et al.* 1989), respectively. The activation of the insulin/IGF-1 signaling pathway depends upon the nutritional environment (Britton *et al.* 2002; Ikeya *et al.* 2002). In addition, nutrition has long been known to affect egg production in many insects (Rivero *et al.* 2001).

In Drosophila oogenesis, yolk protein (YP) synthesis and egg production are related to the nutritional status of the female (Schwartz *et al.* 1985; Bownes and Blair 1986; Bownes *et al.* 1988; Bownes 1989; Bownes and Reid 1990; Søndergaard *et al.* 1995). YPs are synthesized in the fat body and transported from the fat body to the oocyte via the hemolymph (Dimario and Mahowald 1986). In addition, a significant quantity of YP is synthesized in the follicle cells of the ovary (Brennan *et al.* 1982; Isaac and Bownes 1982). The *yolk protein* genes (*yp*'s) are transcribed in the follicle cells from stage 8 of oogenesis (Bownes 1986). Female mosquitoes require a blood meal to produce mature oocytes (Cole and Gillett 1979). A blood meal induces the transcription of vitellogenin, a major yolk protein precursor in the fat body (Kokoza *et al.* 2001). The nutritional environment modulates the hormonal status of the insect; for example, a blood meal in the mosquito causes the ovary to produce ecdysone, which stimulates the fat body to synthesize vitellogenin (Kokoza *et al.* 2001).

Ecdysteroid concentrations are higher in flies under nutritional shortage than in the flies that are supplied with a protein diet and increasing the ecdysone concentration in flies reduces egg production (Bownes 1989). One such hormone, 20-hydroxyecdysone (20E), induces resorption of nurse cells that are degraded by apoptosis in early vitellogenic egg chambers (Soller *et al.* 1999). Normally, apoptosis is not induced in nurse cells until late in oogenesis as part of the program that causes the nurse cells to degenerate once they have fulfilled their function (Foley and Cooley 1998). However, in flies that have 20E at physiological concentrations injected into their abdomen, apoptosis is observed much earlier, at stage 9 of oogenesis (Soller *et al.* 1999). Simultaneous application of a juvenile hormone analog (JHA) suppresses apoptosis of the stage 9 egg chamber (Soller *et al.* 1999). Thus as well as nutrition regulating egg production, the balance between ecdysone and juvenile hormone (JH) in the fly also affects this process and is therefore likely to be crucial in controlling the genetic response of the ovarian cells to nutritional signals.

Ecdysone functions through the ecdysone/ultraspiracle nuclear receptor complex. The complex directly regulates early ecdysone response genes, such as the *Broad-Complex* (*BR-C*), *E74*, and *E75*. The *BR-C* encodes a family of zinc-finger transcription factors (DiBello *et al.* 1991). Apoptosis in many tissues and glands in insects is induced by 20E particularly during morphogenesis and is regulated by ecdysone response genes (Buszczak and Segraves 2000). The *BR-C* is required for transcription of the cell death genes *reaper* (White *et al.* 1996) and *hid* (Abbott and Lengyel 1991) in Drosophila salivary glands (Jiang *et al.* 2000).

In this article, we investigate the choices between vitellogenesis and apoptosis in relation to the nutritional environment during oogenesis and the role of the *BR-C* in this decision. Under conditions of nutritional shortage, such as when flies are grown only on sugar, some oocytes are degenerated by premature apoptosis at stage 8 or 9 of oogenesis. We propose that at stage 8/9 there is a selection between the expression of the apoptosis

[1]*Corresponding author:* Institute of Cell and Molecular Biology, University of Edinburgh, Darwin Bldg., King's Bldgs., Mayfield Rd., Edinburgh EH9 3JR, United Kingdom. E-mail: mary.bownes@ed.ac.uk

genes and the *yp* genes needed for vitellogenesis to proceed, *i.e.*, a choice between the development of an egg and apoptosis of an egg chamber. We show that these developmental choices are controlled by different expression patterns of *BR-C* isoforms. We demonstrate that the nutritional environment of the female affects *BR-C* expression. As a result, the cells in each individual egg chamber become committed either to apoptosis or to progress through normal development. By executing this decision, the flies regulate the number of eggs produced in relation to food.

MATERIALS AND METHODS

Drosophila maintenance: Flies were maintained on standard yeast, maize meal, sugar, and agar medium at 25°. The wild-type strain, Oregon-R, was used throughout. Three-day-old flies were transferred from a standard diet to one with sugar (starved, 1% agar medium, which contains 5% sucrose and 0.005% of a 10% nipagin solution in 95% ethanol) or one with yeast (fed, 2 g baker's yeast on ~50 ml 1% agar medium, which contains 2.5% corn flour, 5% sucrose, 1.75% lypophilized yeast, and 0.005% of a 10% nipagin solution in 95% ethanol). After 3 days on sugar or yeast, flies were dissected (starved, S3; fed, F3), transferred to sugar for 1 day after 3 days on yeast (F3S1), or topically treated with JHA methoprene (ZR515, Zoecon) and maintained on sugar and water for 1 day (F3JHS1), injected with 20E (Sigma, St. Louis), and maintained on yeast for 1 day (F3EF1). We used *BR-C* transgenic flies, which are *TN-Q^1-Q^2-Z1*, *Z2*, *Z3*, and *Z4* (kindly provided by C. Bayer; Bayer *et al.* 1997). The flies were maintained at 25° for 3 days with yeast and underwent heat shock at 39° for 30 min and were maintained at 25° for 6 hr.

Injection of 20E and application of JHA: 20E was dissolved in insect Ringer's solution (130 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$) and 50 nl was injected at a concentration of 2 μg/ml (Soller *et al.* 1997). The concentration of 20E was determined according to Bownes (1989). With a hemolymph volume of ~1 μl/female (Soller *et al.* 1997), 100 pg 20E/female leads to a concentration of 2×10^{-7} M. Methoprene was applied topically to the ventral abdomen in 100 nl acetone. Methoprene diluted 1:100 in acetone corresponds to a concentration of ~1 μg/100 nl. Controls were undertaken injecting Ringer's only and treating flies with acetone.

Hoechst staining and *in situ* hybridization: Hoechst staining has been described by Soller *et al.* (1999) with some modifications. Ovaries were fixed in 4% *p*-formaldehyde in PBS. After fixation, the ovaries were stained in 1 μg/ml Hoechst and the samples were observed using fluorescein filters. The hybridization probes, *BR-C* core, *Z1*, *Z2*, Z3, and *Z4* were labeled with digoxigenin (DIG) as described by the supplier (Boehringer Mannheim Biochemicals).

The protocol is based on the procedure previously described (Tautz and Pfeifle 1989) and modified as follows. The ovaries were dissected in Ringer's solution and fixed for 20 min in 4% *p*-formaldehyde in PBS. After rinsing the tissue in PBT (0.1% Tween-20 in 1× PBS), it was treated for 10 min in methanol/0.5 M EGTA, pH 8 (9:1). The ovaries can then be stored in methanol at −20° for several months. The stored ovaries were rehydrated in PBT. The prehybridization was carried out for 1 hr at 45° in DNA hybrix (50% deionized formamide, 5× SSC, 100 μg/ml sonicated salmon sperm DNA, 50 μg/ml heparin, 0.1% Tween 20). The ovaries were hybridized overnight at 45° in DNA hybrix containing digoxigenin-labeled probe (DIG-DNA labeling and detection kit, Boehringer Mannheim, Indianapolis). For detection a 1:1000 dilution of anti-DIG-alkaline phosphatase (AP)-conjugated Ab was used. The staining reaction was performed in 100 mM Tris pH 9.5, 50 mM MgCl, 10 mM NaCl, 0.2% Tween 20, 8 mM levamisole, 4.5 μl/ml 4-nitro blue tetrazolium chloride, and 3.5 μl/ml X-phosphate (Boehringer Mannheim). Anti-DIG-AP conjugate was preabsorbed with postfixed wilk-type (Oregon-R) ovaries at 4° overnight. The ovaries were mounted in a mixture of PBS/glycerol (1:4) for microscopy. Egg chambers were staged according to size and morphology (King 1970).

RNA extraction and RT-PCR: The levels of *yp*'s and *BR-C* transcripts in ovaries were detected by reverse transcriptase (RT)-PCR as described previously (Hodgetts *et al.* 1995). The details of total RNA extraction, reverse transcription reactions, and the PCR reaction are described in Tzolovsky *et al.* (1999). The expression levels were quantified by National Institutes of Health image (http://rsb.info.nih.gov/nih-image/download.html).

RESULTS

Egg-chamber progression differs between fed and starved flies: Drosophila ovaries develop differently in the fed flies maintained on yeast and in the starved flies maintained on sugar. The fed (F3) flies had more egg chambers than the starved (S3) flies (Figure 1A). In particular, the number of chambers at stages 8 and 9 were significantly different (Figure 1A). This development was also observed in the ovaries of flies that were transferred to a starvation regime after being fed with yeast for 3 days (Figure 1B). In these flies, the number of egg chambers at stages 8 and 9 decreased as the period of starvation was extended (Figure 1B).

A reduction in the number of egg chambers at stages 8 and 9 is also induced by injection of 20E and this reduction is inhibited by application of a JHA (Soller *et al.* 1999). As shown in Figure 1C, if flies were fed for 3 days and then starved for 1 day with JHA, the number of stage 8 and stage 9 egg chambers increased. There is no significant difference in the number of stage 8 egg chambers between control and JHA-treated flies. However, at stage 9 there was a significant rescue by JHA treatment (Figure 1C).

There were large differences in the number of egg chambers at stages 8, 9, and 10 when control (F3) and 20E-injected (F3EF1) flies were compared (Figure 1D). The reduction in stage 8 and 9 egg chambers was greatly increased in 20E-injected flies (Figure 1D).

The reduction in egg chambers at stages 8 and 9 is caused by apoptosis: Nuclear condensation and nuclear fragmentation were observed in the nurse cells following Hoechst staining (Figure 2A), consistent with the reduction in the number of stage 8–10 egg chambers in starved and 20E-injected flies being the result of apoptosis. The proportion of egg chambers undergoing apoptosis at stages 8 and 9 was increased in starved and 20E-injected flies (Figure 2B). In contrast, the proportion of egg chambers undergoing apoptosis was decreased in the JHA-treated flies (Figure 2B).

These results indicate that 20E and JHA had opposite

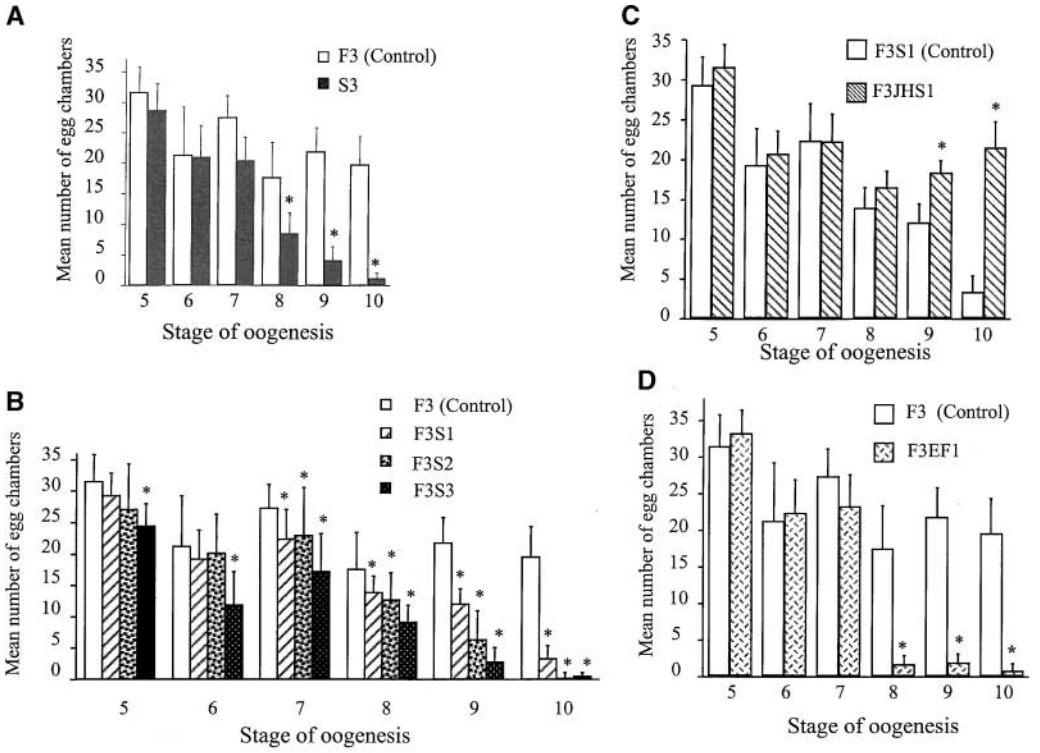

FIGURE 1.—Changes in the distribution and appearance of egg chambers during starvation. (A–D) The number of egg chambers at each stage of oogenesis. The bars represent the mean number ±SD in the flies at each stage under various conditions. $n = 12$ flies. (A) Comparison between fed flies (F3: with yeast for 3 days) and starved flies (S3: without yeast for 3 days). (B) Comparison of fed flies (F3) and flies with various periods of starvation (starved flies: S3 and F3S1, F3S2, F3S3; starved for 1, 2, and 3 days, respectively, after maintaining with yeast for 3 days). (C) The effect of the application of JHA (1 μg applied). Flies were maintained on yeast for 3 days and treated with JHA and flies were maintained without yeast (starved, F3JHS1). (D) The effect of the injection of 20E (100 pg injected). Flies were maintained on yeast for 3 days, injected with 20E, and retained on yeast (F3EF1). (*) Significant differences within a 5% level.

effects on the progression of oogenesis in fed (F3) and starved (S3, F3S1∼3) flies. Furthermore, the difference in the numbers of normal egg chambers under specific nutritional or hormonal conditions was caused by apoptosis. Apoptosis was induced by 20E and inhibited by JHA.

***BR-C* expression is altered in ovaries undergoing apoptosis at stages 8 and 9:** One of the ecdysone-responsive genes, *BR-C*, plays an important role in egg-chamber development (DENG and BOWNES 1997). We therefore determined the spatial and temporal expression of *BR-C* to examine if the expression pattern was altered with changes in nutrition or hormone level.

Expression of *BR-C* was first detected in all follicle cells at stage 5 of oogenesis and was stronger than that at stage 6 (Figure 3, A, F, and H). Strong *BR-C* expression in the starved flies (S3 and F3S1) was first detected in follicle cells at stage 6 (Figure 3, B, C, and I), and *BR-C* expression at stage 5 either was not detected or was very faint (Figure 3G). However, when JHA was applied to the abdomen of starved flies, *BR-C* expression was induced in the follicle cells at stage 5 and to a higher level than in cells at stage 6 (Figure 3E). Thus in starved flies, JHA treatment induced a *BR-C* expression pattern that was similar to that of fed flies. In contrast, when 20E was injected into the abdomen of fed flies, strong *BR-C* expression was induced in follicle cells at stage 6 (Figure 3D), and the *BR-C* expression pattern in 20E-injected flies was similar to that of starved flies. Thus the timing of expression of *BR-C* in follicle cells is altered according to the nutritional conditions and in response to hormones.

***BR-C* transcripts encoding different isoforms show different expression patterns:** *BR-C* has four isoforms, *Z1*, *Z2*, *Z3*, and *Z4* (DIBELLO *et al.* 1991). The overall distribution of *BR-C* mRNA was detected by a core *BR-C* probe (this detects a common region in all splice variants). In addition, specific probes were used to detect the RNA that would encode each isoform separately. Figure 4A shows the proportion of *BR-C* expression in the egg chambers at stages 5 and 6 in flies under various experimental conditions. The proportion of *Z1* expression at stage 5 is almost the same as the overall *BR-C* expression (core); however, the proportion of the expression at stage 6 was substantially lower. *Z2* and *Z3* were expressed at stage 5 in similar proportions to each other in fed flies. *Z2* expression at stage 6 was not induced in fed flies and was reduced by JHA treatment. *Z3* expression at stage 6 was constant under all experimental conditions. *Z4* expression at stages 5 and 6 was very weak; this gene was expressed at lower levels than the other isoforms.

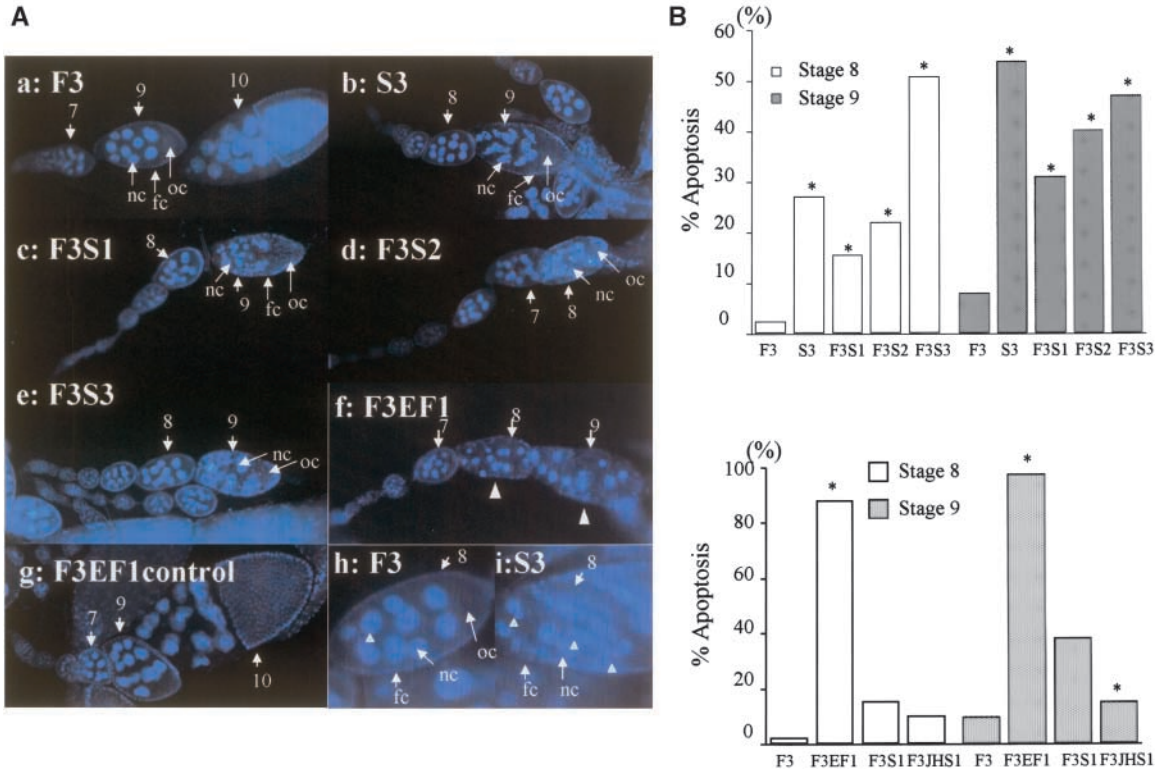

FIGURE 2.—Nuclear condensation and fragmentation after starvation. (A, a–i) The nuclei of ovaries were stained with Hoechst. (a) Ovariole and (h) detail of stage 8, F3; (b) ovariole and (i) detail of stage 8, S3; (c) F3S1; (d) F3S2; (e) F3S3; (f) F3EF1; (g) F3EF1 control, maintained on yeast for 3 days, injected with 50 nl Ringer's solution as control F3EF1 (f). nc, nurse cell; fc, follicle cell; oc, oocyte. Each number indicates the stages of oogenesis. Arrowhead in h shows the nucleus of the nurse cell and arrowhead in i shows the nuclei of nurse cells that are undergoing nuclear condensation or fragmentation. (B) The effects of starvation, JHA application, and 20E injection. The bars represent the mean number at each stage in the flies in various conditions. *y*-axis indicates the percentage of the apoptosis-induced egg chambers (n = 12 flies). The egg chambers were counted with a fluorescent microscope. (*) Significant differences within 5% level.

Stage 8 in oogenesis is an important stage in development. YP synthesis starts at this stage in the follicle cells of egg chamber (WILSON 1982; BOWNES 1986; SOLLER *et al.* 1999), and uptake of yolk from the hemolymph begins. Starvation and 20E injection induces apoptosis at this stage (Figure 2). We therefore assume that at this point there should be a key developmental decision in oogenesis. *BR-C* and *Z1* expression at stage 8 was almost the same under all experimental conditions and *Z4* was not detected at this stage (Figure 4B). The proportion of the *Z2* and *Z3* expression increased in the egg chambers of starved and fed, 20E-treated flies, namely under conditions that induce apoptosis (Figure 4B). These results suggest a key role for *Z2* and *Z3* in regulating the apoptosis switch.

***BR-C Z3* activates *Z2* expression in fed condition:** Previously, it has been shown that *BR-C* isoforms interact with each other to control gene expression and tissue differentiation in response to ecdysone during Drosophila metamorphosis (MUGAT *et al.* 2000). In third instar larvae, *Z3* antagonizes *Z2* repression and primes enhancer activation (MUGAT *et al.* 2000). As the hormone titer rises in late third instar, the ligand-bound ecdysone receptor no longer silences gene expression and, together with the *Z1*, *Z3*, and *Z4 BR-C* isoforms, activates transcription (MUGAT *et al.* 2000).

Since it seemed possible that *BR-C* isoforms suppress or enhance the expression of other isoforms, we investigated the effect of the *BR-C* isoform overexpression on the expression of other isoforms. In fed flies transgenic for *Z3*, not only *Z3* but also *Z2* expression was increased in the ovary when *Z3* was activated (Figure 5A). In starved flies, however, *Z3* did not activate *Z2* expression in the ovary. We found that *Z2* expression at stage 8 was activated by heat shock of *Z3* transgenic flies under adequate nutritional conditions, but was not activated without heat shock in control flies. The level of *Z2* expression in heat-shocked *Z3* transgenic flies increased compared with non-heat-shocked *Z3* transgenic flies (Figure 5B). These results indicate that *Z3* overexpression induces *Z2* expression in fed flies but not in starved flies.

***BR-C* regulates *YP* gene expression:** Two key choices are made at stage 8 of oogenesis: the start of YP synthesis or the induction of apoptosis. *BR-C Z2* and *Z3* had a role in the induction of apoptosis at stages 8 and 9

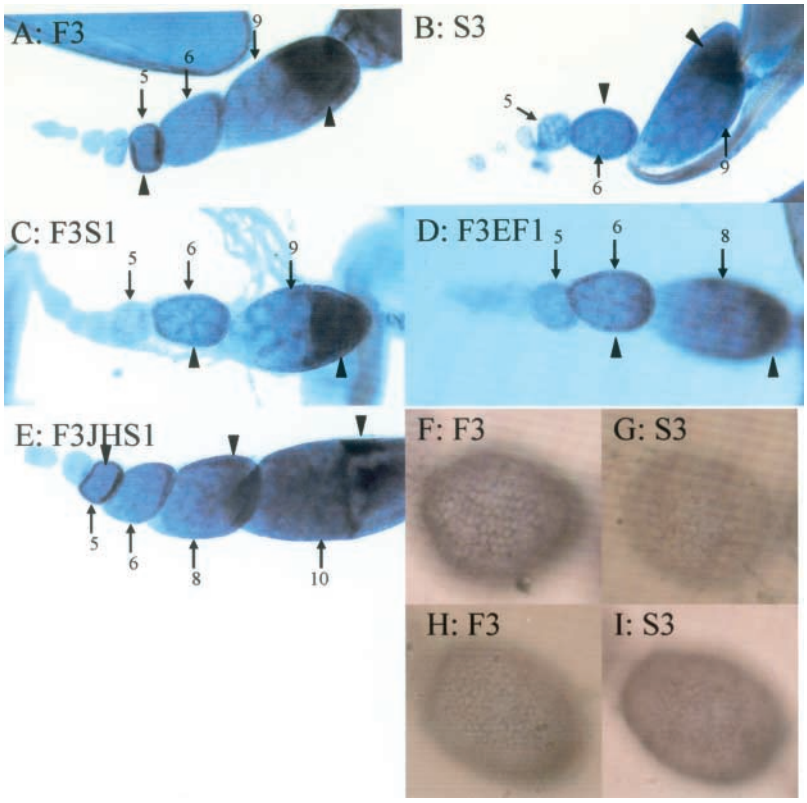

FIGURE 3.—*BR-C* expression under each experimental regime and the effects of 20E injection and JHA application. (A) F3, (B) S3, (C) F3S1, (D) F3EF1, (E) F3JHS1, and (F and G) enlarged egg chambers at stage 5 in fed and starved flies, respectively. (H and I) Enlarged egg chambers of stage 6 in fed and starved flies, respectively. Each number indicates the stage of oogenesis. Arrowhead shows *BR-C* expression.

(Table 1). So we next examined the role of the *BR-C* in regulating *yp1* expression.

yp1 gene expression was inhibited by starvation in F3S1 flies, but not in S3 flies (Figure 6A) and JHA treatment rescued *yp1* expression in starved flies. 20E did not affect *yp1* expression (Figure 6A). *yp2* and *yp3* expression were suppressed in both starved flies, F3S1 and S3, and also rescued by JHA treatment. 20E injection into the fed flies resulted in suppression of *yp2* and *yp3* expression.

BR-C Z1 and *Z2* overexpression also affect *yp* expression in fed and starved conditions (Figure 6B). *Z1* and *Z3* overexpression suppressed *yp1*, *yp2*, and *yp3* expression in both fed and starved flies, but *Z2* and *Z4* did not affect *yp* expression under any conditions.

DISCUSSION

A poor nutritional environment induces apoptosis in the egg chamber at stages 8 and 9: In Drosophila oogenesis, the nutritional status of the female's environment affects YP synthesis and egg production (SCHWARTZ *et al.* 1985; BOWNES and BLAIR 1986; BOWNES *et al.* 1988; BOWNES 1989; BOWNES and REID 1990; SØNDERGAARD *et al.* 1995). We have shown that stage 8 and 9 egg chambers in starved flies are degenerated by apoptosis, leading to a reduced number of egg chambers beyond stage 8. Hence fewer eggs are laid (Figures 1 and 2). During nutritional shortage, the nutrients must be used to develop a smaller number of normal eggs. To achieve this, it is essential to select which egg chambers will develop to mature eggs and which will die.

When the ovary accumulates stage 14 oocytes in the sexually mature virgin female, the production of new eggs is prevented by resorption of egg chambers at stage 9, mediated by the high level of 20E in the hemolymph (BOWNES 1989; HARSHMAN *et al.* 1999; SOLLER *et al.* 1999). It seems likely that the resorption of stage 9 egg chambers under nutritional stress may well occur by a similar mechanism, since reduced food induces increased ecdysone concentration in the flies (BOWNES 1989), and 20E in turn induces apoptosis of the egg chambers at stages 8 and 9. While ecdysone induces a reduction in the number of egg chambers, JH acts to suppress this reduction (SOLLER *et al.* 1999 and this article). Females homozygous for ap^{56f} produce low levels of JH but are fertile although vitellogenesis is subject to a delay (ALTARATZ *et al.* 1991). The ap^{56f} ovaries are capable of ultimately producing greater than wild-type amounts of ecdysteroids (RICHARD *et al.* 1998). It is possible that the ecdysone concentration in the flies under starvation is suppressed when JHA is applied to the abdomen of the flies and thus JHA suppresses the ecdysone-induced apoptosis in egg chambers at stage 8/9. It is likely that both ecdysone and JH levels are affected by the nutritional status of Drosophila. Ecdysone and JH concentration could be modulated by synthesis of hormones or by inducing metabolic pathways

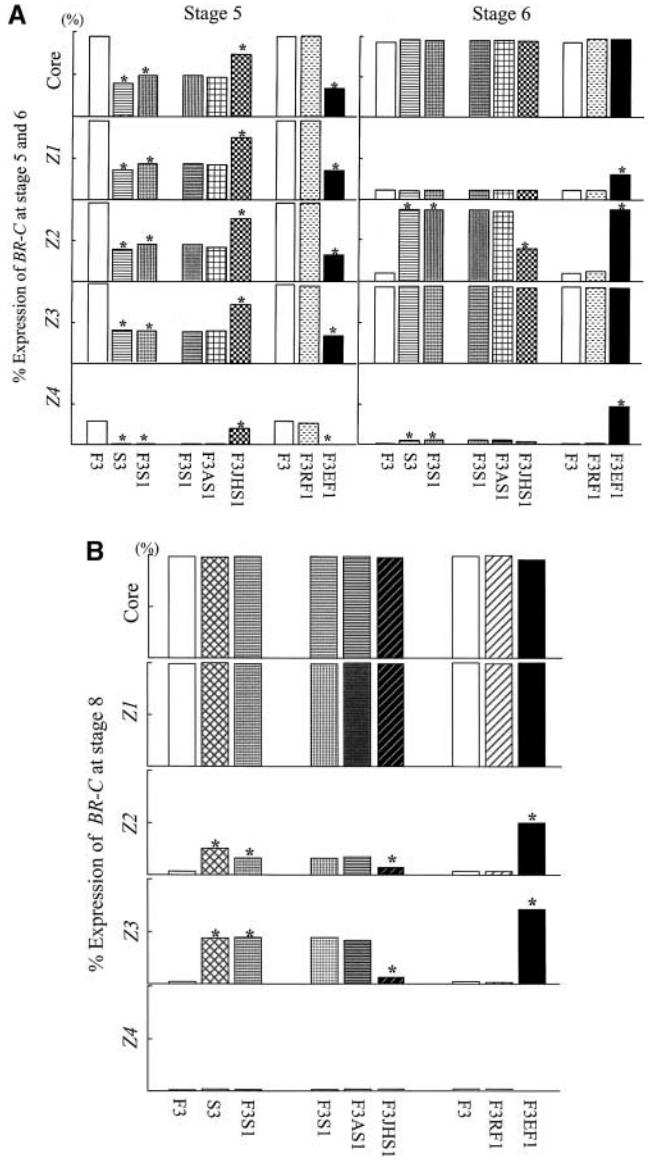

FIGURE 4.—The expression of *BR-C* isoforms at stage 5, 6, and 8 in various conditions. The graphs show the percentage of egg chambers expressing each isoform of *BR-C* at stages 5 and 6 (A) and at stage 8 (B). The expression signals were detected by *in situ* hybridization for using each probe, core (common region of *Z1–Z4*), *Z1*, *Z2*, *Z3*, and *Z4*. The flies called F3AS1 were maintained on yeast for 3 days, treated with 100 nl acetone, and maintained 1 day without yeast as a control for F3JHS1. The flies of F3EF1 were injected with 50 nl insect Ringer's and maintained on yeast for 1 day as a control for F3EF1. The values were calculated as percentages (mean of egg chambers, expressed/mean of egg chambers in each ovary × 100; n = 12 flies). (*) Significant differences compared to the control (F3 *vs.* F3S1, F3 *vs.* F3EF1, and F3S1 *vs.* F3JHS1) within 5% level.

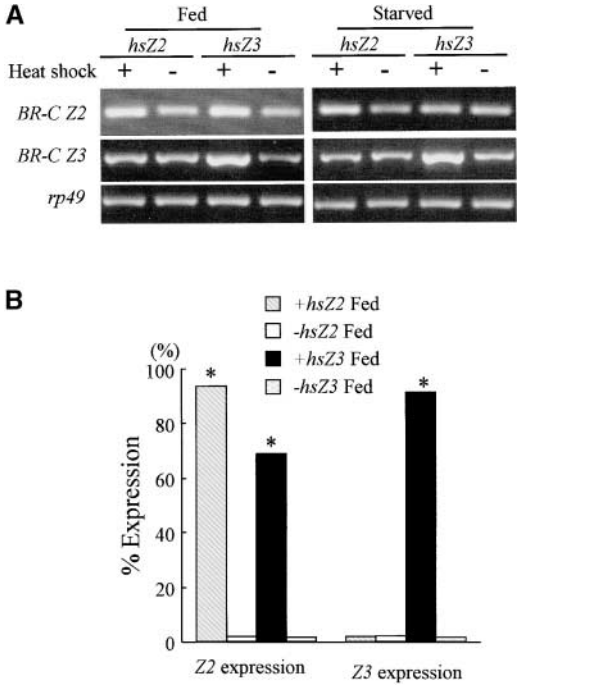

FIGURE 5.—*BR-C Z3* overexpression induces *BR-C Z2* expression at stage 8. (A) The result of RT-PCR in the ovaries of transgenic flies *hsZ2* and *hsZ3*. *rp 49* is control. (B) The percentage of *Z2* and *Z3* expression at stage 8 in *hsZ2* and *hsZ3* flies. The expression signals were detected by *in situ* hybridization. *y*-Axis indicates the percentage of the egg chambers in which *Z2* or *Z3* is expressed (n = 12 flies). (*) Significant differences within 5% level.

that degrade the hormones. Using this mechanism a female can relate the number of eggs produced to the food available.

***BR-C* is the key gene for selecting between apoptosis and development:** *BR-C* isoforms and *BR-C* mRNA are expressed differentially in a number of tissues during metamorphosis (HUET *et al.* 1993; EMERY *et al.* 1994) and control subsequent cell differentiation. Late in Drosophila oogenesis, *BR-C* regulates the dorsal-ventral signaling pathway (DENG and BOWNES 1997) and leads to prolonged endoreplication of follicle cell DNA and to additional amplification of specific genes (TZOLOVSKY *et al.* 1999). The *BR-C* expression pattern differs between starved and fed flies earlier in oogenesis at stages 5, 6, and 8 (Figures 3 and 4). *BR-C* expression in oogenesis is first detected in the egg chamber at stage 5 under normal nutritional conditions (Figure 3), but under nutritional shortage it is detected later, at stage 6 (Figure 3). This shows that nutritional shortage is detected in the follicle cells of the egg chamber in previtellogenic stages. During metamorphosis of holometabolous insects, the developmental program is switched from "larval" to "pupal" during the final larval instar, a switch that is referred to as pupal commitment (RIDDIFORD 1985). To execute apoptosis, tissues and glands must first become committed to apoptosis and then execute the decision and die. We propose that the altered timing of *BR-C* expression affects this commitment stage in the egg chamber. By stage 8, when the egg chamber must develop or die, the different isoforms of *BR-C*, specifically *Z2* and *Z3*, differ between starved and fed flies. *Z2* and *Z3* are not expressed in egg chambers at stage

TABLE 1

Percentage of egg chambers showing apoptosis at stages 8 and 9 in the ovary of flies misexpressing *BR-C* isoforms

		Fed (F3)				Starved (F3S1)			
		+hs Stage 8	−hs Stage 8	+hs Stage 9	−hs Stage 9	+hs Stage 8	−hs Stage 8	+hs Stage 9	−hs Stage 9
Control	OrR	2.2	1.7	5.0	4.7	16.2	14.9	28.0	29.2
BR-C	*Z1*	3.8	1.8	8.4	4.8	16.6	15.6	28.1	23.3
	Z2	2.2	2.2	10.8*	3.2	30.6*	14.8	44.6*	22.8
	Z3	4.6	2.3	33.1*	3.3	32.1*	16.4	46.1*	28.4
	Z4	2.8	3.4	5.0	5.1	16.6	18.0	27.7	28.5

The percentage of apoptosis at stages 8 and 9 in ovaries of heat-shock transgenic flies for *BR-C* isoforms with and without heat shock is shown. Heat-shock flies underwent heat shock at 39° for 30 min and were maintained at 25° for 6 hr with yeast prior to dissection. Nuclear condensation and fragmentation were detected by Hoechst staining and observed under a fluorescent microscope. The fed flies (F3) were maintained 3 days with yeast, and the starved flies (F3S1) were maintained for 1 day of starvation after maintaining them for 3 days with yeast. We used *BR-C* transgenic flies that are *TN-Q*^{1}*-Q*^{2}*-Z1*, *Z2*, *Z3*, and *Z4* (construction is described in Bayer *et al.* 1997). An asterisk indicates significant differences within 5% (n = 12 flies). −hs, without heat shock; +hs, with heat shock.

8 under normal nutritional conditions, but they are expressed in egg chambers at stage 8 under nutritional shortage or if 20E is injected into fed flies (Figure 4B). This means that *Z2* and *Z3* are expressed under apoptotic conditions. Furthermore, *Z2* and *Z3* overexpression induces apoptosis at stages 8 and 9 (Table 1).

In the ovary of starved flies, *Z2* and *Z3* expression is independent, but in fed flies *Z3* overexpression induces *Z2* expression (Figure 5). 20E injection overrides the nutritional signals and induces apoptosis in the egg chambers at stages 8 and 9 in fed flies (Figure 2) probably by activating *Z3* that in turn induces *Z2* expression. *Z3* therefore acts as key transcript for inducing apoptosis. On the basis of these results, *BR-C* acts as a selector gene for progressing oogenesis or inducing apoptosis at stages 8 and 9.

The nutritional environment affects oogenesis by regulating *BR-C* expression: Figure 7 shows a model for the control of oogenesis in relation to the nutritional status. Each Drosophila egg chamber selects between two fates

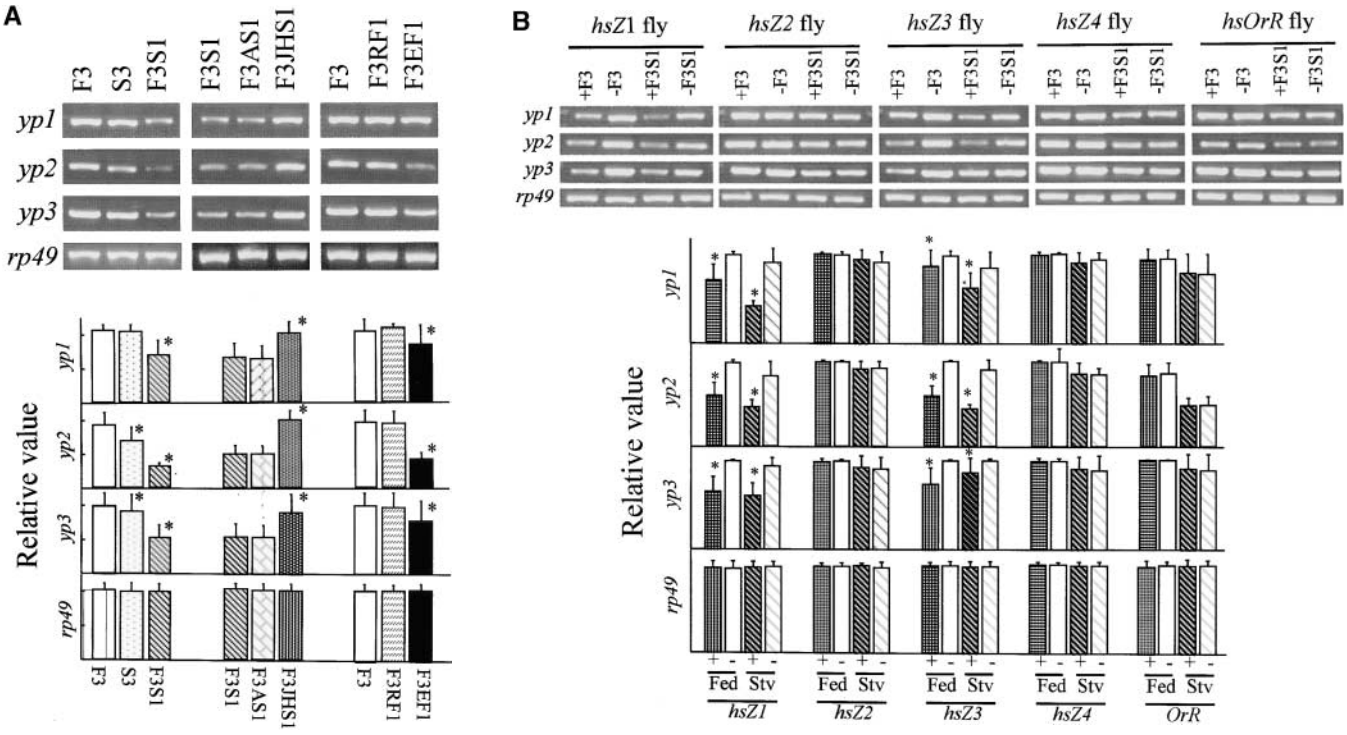

Figure 6.—Nutritional conditions and *BR-C* overexpression affect *YP* gene expression in the ovary. (A) Starvation, JHA application, and 20E injection affect *YP* gene expression in the ovaries. The graph indicates the expression level using relative value with ±SD (measured by National Institutes of Health image). (B) The *BR-C* isoform overexpression affects *YP* gene expression in the ovary. An asterisk indicates significant differences within 5%.

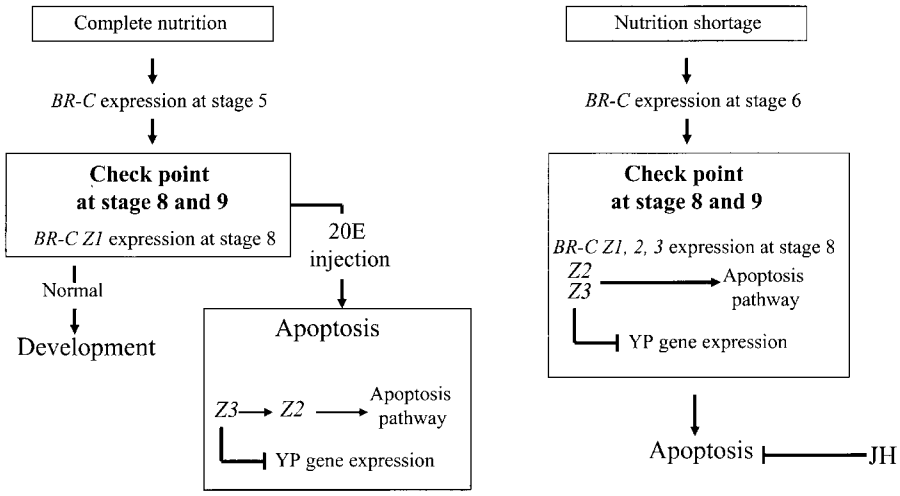

FIGURE 7.—The role of *BR-C* in oogenesis progression. Complete nutrition induces normal development of the mature egg during oogenesis. In this case, only *BR-C Z1* is expressed in the egg chamber at stage 8. But if there are problems during oogenesis, or if 20E is injected, the apoptosis pathway is activated and apoptosis is executed at stages 8 and 9. Nutritional shortage induces apoptosis commitment and the progression of apoptosis. In this case, not only *Z1* but also *Z2* and *Z3* are expressed in the egg chamber at stage 8. This apoptosis is suppressed by application of JHA onto the abdomen.

during development. These fates are triggered by the hormonal conditions that are in turn regulated by the nutritional environment. The higher ecdysone concentration in starved flies induces *BR-C Z2* and *Z3* expression at stage 8, and these genes activate the pathway of apoptosis in the egg chambers at stages 8 and 9. In fed flies, the ecdysone concentration is lower and hence *Z2* and *Z3* are not expressed in the egg chambers at stage 8. The JH analog, methoprene, suppresses apoptosis at stages 8 and 9, suggesting that JH acts as a suppressor of apoptosis and an inducer of normal development. The normal developmental pathway requires that *yp* genes are expressed by stage 8; however, *Z3* suppresses *yp* expression (Figure 7). Thus *yp* expression is suppressed by nutritional shortage and by 20E injection in fed flies. The expression of *Z3* that is induced by nutritional shortage or 20E injection suppresses *yp* expression at stage 8 in the follicle cells and hence prevents normal development from continuing.

In summary, we have shown that the environmental status of the fly is sensed by each individual egg chamber and that some develop and some die in proportion to the food available. The nutritional status of the environment controls the developmental fate of the egg chamber in Drosophila by adjusting the hormonal balance in the fly. We have established that the key gene in follicle cells that is controlled by the hormone balance is *BR-C* and that the *BR-C Z3* isoform particularly is important for egg-chamber development in Drosophila in relation to the nutrition available.

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