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We studied the regulation of the yolk protein (YP) genes in the somatic cells of the gonads, using temperature sensitive mutations (tra-2ts) of transformer-2, a gene required for female sexual differentiation. XX;tra-2ts mutant animals were raised at the permissive temperature so that they developed as females and were then shifted to the restrictive male-determining temperature either 1-2 days before or 0-2 h after eclosion. These animals formed vitellogenic ovaries. Likewise, mutant gonads transplanted into either normal female hosts or normal male hosts, kept at the restrictive temperature, underwent vitellogenesis. Thus, the ovarian follicle cells can mature and express their YP genes in the absence of a functional product of the tra-2 gene. Although the gonadal somatic cells of ovary and testis may derive from the same progenitor cells, the testicular cells of XX;tra-2ts pseudomales did not express their YP genes nor take up YP from the haemolymph at the permissive female-determining temperature. We conclude that in the somatic cells of the gonad, the YP genes are no longer under direct control of the sex-determining genes, but instead are regulated by tissue specific factors present in the follicle cells. It is the formation of follicle cells which requires the activity of tra-2.

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Differential control of yolk protein gene expression in fat bodies and gonads by the sex-determining gene *tra-2* of *Drosophila*

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We studied the regulation of the yolk protein (YP) genes in the somatic cells of the gonads, using temperature sensitive mutations (*tra-2*^{ts}) of *transformer-2*, a gene required for female sexual differentiation. *XX;tra-2*^{ts} mutant animals were raised at the permissive temperature so that they developed as females and were then shifted to the restrictive male-determining temperature either 1-2 days before or 0-2 h after eclosion. These animals formed vitellogenic ovaries. Likewise, mutant gonads transplanted into either normal female hosts or normal male hosts, kept at the restrictive temperature, underwent vitellogenesis. Thus, the ovarian follicle cells can mature and express their YP genes in the absence of a functional product of the *tra-2* gene. Although the gonadal somatic cells of ovary and testis may derive from the same progenitor cells, the testicular cells of *XX;tra-2*^{ts} pseudomales did not express their YP genes nor take up YP from the haemolymph at the permissive female-determining temperature. We conclude that in the somatic cells of the gonad, the YP genes are no longer under direct control of the sex-determining genes, but instead are regulated by tissue specific factors present in the follicle cells. It is the formation of follicle cells which requires the activity of *tra-2*.

Key words: Drosophila / gonads / sex determination / transformer-2/yolk proteins

Introduction

In *Drosophila*, sexual differentiation of somatic cells is controlled by a small number of regulatory genes (*liz*, *Sxl*, *vir*, *tra*, *tra-2*, *ix* and *dsx*) that are arranged in a hierarchical manner (for review see Baker and Belote, 1983; Nöthiger and Steinmann-Zwicky, 1985; Wolfner, 1988; Baker, 1989; Steinmann-Zwicky *et al.*, 1990; Slee and Bownes, 1990). Ultimately, all aspects of somatic sexual differentiation depend on the mode of expression of the bifunctional gene *dsx* at the end of the cascade (Baker and Ridge, 1980; Nöthiger *et al.*, 1987). This gene produces a female-determining protein (DSX^F) if *Sxl*, *vir*, *tra* and *tra-2* are active; if any one of these four genes is inactivated by mutation, *dsx* expresses the male-determining function (DSX^M) so that XX animals are transformed into phenotypical males, so-called pseudomales (for review see Steinmann-Zwicky *et al.*, 1990).

In somatic cells, the overt sexual phenotype is brought about by the sex differentiation genes which are somehow controlled by the regulatory genes, i.e. eventually by *dsx*. The mechanism by which these terminal differentiation genes are regulated is largely unknown. The discovery of temperature sensitive alleles of *tra-2* (*tra-2*^{ts}) which render sexual development temperature dependent (Belote and Baker, 1982, 1983) has helped to reveal, at least in principle, the strategy by which the regulatory genes achieve concrete sexual differentiation.

The sex differentiation genes can be under direct and continuous control by the regulatory genes; alternatively, their regulation can be delegated to the cell type (for review see Nöthiger and Steinmann-Zwicky, 1987; Wolfner, 1988; Steinmann-Zwicky *et al.*, 1990; Slee and Bownes, 1990). The synthesis of yolk proteins (YP) in the fat body of females is an example of the first type. In XX animals that are mutant for *tra-2*^{ts}, the YP genes can be turned on and off at will by shifting adult flies (pseudomales or females) forth and back between the permissive and the restrictive temperature (Belote *et al.*, 1985; Bownes *et al.*, 1987). In contrast, the transcription of genes coding for male specific transcripts (*mst*) of yet undefined functions in the male accessory glands (paragonia) does not cease when *tra-2*^{ts} pseudomales are shifted to the permissive, i.e. female-determining temperature (Schäfer, 1986a,b; DiBenedetto *et al.*, 1987; Chapman and Wolfner, 1988). In this tissue, the differentiation genes no longer react to changes in the activity of the sex-determining genes, but have become independent of these. Their control has been taken over by the cell type (reviewed by Wolfner, 1988; Steinmann-Zwicky *et al.*, 1990). In this case, the role of *tra-2* is to direct the primordial cells into the appropriate sexual pathway and to create the sex specific cell type in which the synthesis of sex specific products then takes place.

The YP genes which are under the control of the sex-determining genes (Bownes and Nöthiger, 1981) are expressed in the fat body and in ovarian follicle cells of adult females (Bownes and Hames, 1978; Barnett and Wensink, 1981; Isaac and Bownes, 1982; Brennan *et al.*, 1982; Garabedian *et al.*, 1985, 1986). This offers the unique opportunity to study the sex specific regulation of the same genes in two types of tissues. The fat body is a tissue common to both sexes; in it, the YP genes are under continuous control of the sex-determining genes (Belote *et al.*, 1985; Bownes *et al.*, 1987). The ovarian follicle cells, on the other hand, are present only in females. So far, it is not known how the YP genes are regulated in this sex-limited tissue, except that Wensink and his collaborators (Garabedian *et al.*, 1985; Logan *et al.*, 1989; Logan and Wensink, 1990) have identified two *cis* regulatory regions that promote expression of the *yp1* and *yp2* genes in follicle cells. We decided to approach this question by studying the production and uptake of YP in ovaries of *XX; tra-2*^{ts} females at the restrictive temperature which abolishes the

function of the *tra-2* protein and thus dictates maleness.

Another question arises from the different developmental history of accessory glands and follicle cells. The accessory glands which produce the *mst* are part of the male copulatory apparatus. They are male specific organs, with no corresponding (homologous) tissue in females. They are formed by the primordia of abdominal segment 9 which develop in males, but remain repressed in females (Nöthiger *et al.*, 1977; Schüpbach *et al.*, 1978). In contrast, the gonad appears to derive from a bisexual primordium which either forms an ovary or a testis (Gehring *et al.*, 1976; Szabad and Nöthiger, unpublished). Thus, the somatic cells of ovaries and testes may be homologous, but with significant functional differences between the terminally differentiated cell types. The ovarian follicle cells and the testicular epithelial cells, then, may represent a cell type existing in both sexes, but in a different form and performing different functions. We therefore asked if the YP genes can be activated in cells of the testicular epithelium of *XX;tra-2*^{ts} pseudomales and if such cells can be stimulated to take up YP from the haemolymph.

Our experiments address the problem of interaction, or interdependence, of cell type (specified by homeotic selector genes) and sex (specified by *tra-2* and the other sex-determining genes), and how sexual differentiation is ultimately achieved.

Results

Animals of genotype *X/X; tra-2*^{ts}/*tra-2*^{ts} develop into phenotypically normal females at 16°C. When homozygous for the allele *tra-2*^{ts1}, they are sterile even at the permissive temperature. When homozygous for *tra-2*^{ts2} or heterozygous for *tra-2*^{ts1}/*tra-2*^{ts2}, they are weakly fertile at 16°C (Belote and Baker, 1982, 1983). The gonads of these flies are either undifferentiated or they are ovaries in which all stages of oogenesis can be present; the numbers and proportions of cells in each stage are variable. Raised at 29°C, the animals develop into sterile pseudomales with a fat body in which the genes coding for yolk proteins (YP) are essentially inactive (Belote *et al.*, 1985). They have rudimentary testes of variable size, containing mostly abortive germ cells. The two mutations have no effect in somatic cells of XY animals.

Vitellogenesis begins and proceeds in ovaries of X/X; tra-2^{ts} *females at the restrictive temperature*

The ovaries of newly eclosed females do not synthesize nor contain yolk proteins. In wild-type females, raised at 25°C, transcription of the YP genes in the fat body begins immediately after eclosion. In the ovary, they start being transcribed when the first follicles have reached stages 8 which occurs some 4–6 h after eclosion and remain active in these cells until stage 11 some 20 h later. During this period, vitellogenesis (uptake of YP into the growing oocyte) takes place (Bownes, 1982; Brennan *et al.*, 1982).

Homozygous *X/X; tra-2*^{ts} flies were raised at 16°C and shifted to 29°C immediately after eclosion. These females produced YP despite the restrictive temperature. We dissected 66 flies of genotype *X/X; tra-2*^{ts1}/*tra-2*^{ts1} at various times after the temperature shift. Early vitellogenic stages were present after 1 day and mature eggs after 2 days. The mutant females laid no eggs so that even after 6 days, mature eggs were still present in the ovaries. These, however, now contained only few earlier vitellogenic stages, and some early egg chambers showed signs of degeneration. We also analysed 22 females of genotype *tra-2*^{ts1}/*tra-2*^{ts2}. After 10 days at 29°C, most of the ovaries contained eggs and degenerating egg chambers, and some had vitellogenic stages. These experiments thus show that YP are synthesized and incorporated into oocytes at the restrictive temperature, but also that degenerative processes take place.

The YP present in the mutant ovaries were either synthesized by the follicle cells or by the fat body or by both tissues. When *tra-2*^{ts} mutant females were shifted some days after eclosion to 29°C, transcription and translation of the YP genes gradually ceased in the fat body (Belote *et al.*, 1985). Thus, the degenerative processes observed in the ovaries in our experiment could result from insufficient import of YP from the haemolymph into the growing oocytes. To test if mutant ovaries degenerate even in an environment with normal amounts of YP in the haemolymph, they were transplanted into female hosts. The data in Table I demonstrate that the development of *tra-2*^{ts} mutant ovaries is not improved by providing them with YP, and that the eggs are still not deposited, even when the ovary is attached to a set of wildtype gonoducts.

In our first experiment in which we shifted *tra-2*^{ts} mutant females to the restrictive temperature, the fat body still produced traces of YP, even after 3 days at 29°C (data not

Table I. Development of ovaries after transplantation into larval hosts

Genotype of transplanted donor ovary (X/X)	*N*	Hosts with ovaries attached			Hosts with ovaries not attached			No implant found
		Number of flies	Number of eggs laid	Morphology of ovary	Number of flies	Number of eggs laid	Morphology of ovary	
tra-2^{ts2}/*tra-2*^{ts2}	9	3	1	abnormal, degenerate	3	0	abnormal, degenerate	3
tra-2^{ts2}/*T(2;3)ap*^{Xa} (control)	8	4	many	normal	3	0	normal, fewer immature stages	1

Donor larvae from the cross between females and males of *T(2;3)ap*^{Xa}/*tra-2*^{ts2}*bw; st* were raised *ab ovo* at 16°C. Homozygous mutant and heterozygous control larvae were distinguished by the colour of their Malpighian tubules which were white in mutant, yellow in control larvae. Larval ovarian anlagen were transplanted at the end of the third instar into female host larvae of genotype +/*ovo*^{D1}. The hosts were sterile daughters of Oregon R females crossed to *ovo*^{D1}/Y males; such hosts have normal amounts of YP in their haemolymph (M.Bownes, data not shown). After the operation, the animals were maintained at 16°C until 6–7 days after eclosion. They were then shifted to 29°C and kept individually in vials with *cn tra-2 bw/SM5* males. Eight days later, they were dissected to inspect the state of the implanted ovary.
N, number of adult female hosts that survived the operation.

Table II. Development of gonads of *X/X; tra-2*^{ts1}/*tra-2*^{ts1} animals kept at 29°C prior to eclosion

Days spent at 29°C prior to eclosion	Number of flies	Phenotype of genitalia	Flies with 1 or 2 vitellogenic ovaries	Accessory glands	Testes
0–1	27	female	15	0	0
1–3	18	female	6	0	0
	6	intersexual	0	6	0
3–7	11	intersexual	0	3	0
	16	male	0	16	8[a]

The animals were reared at 16°C and shifted to 29°C prior to eclosion.
[a]The testes were rudimentary and not properly coiled as is typical for testes of pseudomales (Nöthiger *et al.*, 1989).

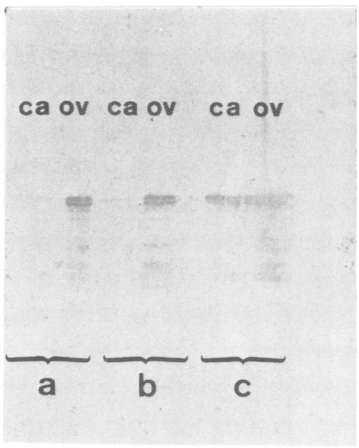

Fig. 1. Ovaries of *X/X; tra-2*^{ts} mutant flies synthesize YP when shifted from 16°C to 29°C prior to eclosion. Animals of the genotype *X/X; tra-2*^{ts1}/*tra-2*^{ts1} were reared at 16°C, and bottles with pupae were then transferred to 29°C. Adult flies were collected daily, having spent 1–2 days (**a**) or 0–1 day (**b**) at 29°C prior to eclosion. The flies were then kept at 29°C for 3–4 more days to allow their ovaries to mature. The two ovaries (ov) of one fly and the castrated bodies (carcasses, ca) of four flies were assayed by Western blotting for the presence of YP, using a polyclonal antibody directed against all three yolk proteins. The carcass and the ovaries of one wild-type female served as a control (c). The bands of lower molecular weight are degradation products of YP.

shown), so that the observed vitellogenesis could have resulted from uptake of YP rather than from endogenous synthesis by the ovary. To minimize the external source of YP, fly cultures were shifted to 29°C at various times prior to eclosion. Table II shows that vitellogenic ovaries, some with mature oocytes of stage 14, could develop under these conditions. The presence of ovaries was negatively correlated with the presence of male sexual characters which appeared with prolonged periods spent at 29°C. Western blots revealed that the ovaries of a single fly contained substantially more YP than four castrated flies (Figure 1). The results strongly suggest that the YP found in the ovaries were synthesized by the follicle cells after the shift to the restrictive temperature although the possibility remains that a residual production of the fat body may have contributed.

To eliminate all external sources of YP, we transplanted immature ovaries of freshly emerged mutant *tra-2*^{ts} females and of control females into adult male hosts which do not contain any YP. The males were put at 29°C immediately after the operation. As shown in Table III, vitellogenic ovaries were again observed. Since transcription of the YP genes in the follicle cells begins only some 4–6 h after eclosion, we conclude that the YP genes, once the ovary is formed, become active even at the restrictive temperature and synthesize YP in follicle cells that presumably no longer have a functional product of *tra-2*. After 10 days, all ovaries contained mature oocytes and most of them also earlier vitellogenic stages. Degenerating egg chambers were sometimes present in experimental and control ovaries, probably as a consequence of high temperature and eggs being retained.

YP synthesis cannot be induced in tra-2^{ts} *pseudomales*

The follicular and testicular epithelia may be homologous. It is thus conceivable that the testicular epithelial cells of *tra-2*^{ts} pseudomales could be stimulated to activate their YP genes or to take up YP from the haemolymph when shifted to the permissive temperature.

Animals were reared at 29°C, and adult *X/X; tra-2*^{ts} pseudomales were collected. These were shifted to 16°C either immediately after eclosion or 2–3 days later. After 4 or 10 days at 16°C, their testes and accessory glands were isolated and assayed for the presence of YP by Western

Table III. Ovaries of mutant *tra-2*^{ts} females, transplanted into adult male hosts, can mature and become vitellogenic at 29°C

Genotype of ovaries	Number of transplanted ovaries	Number not developing	Number with only previtellogenic and immature vitellogenic oocytes	Number with immature and mature vitellogenic oocytes	Number with only mature vitellogenic oocytes and degenerating egg chambers
A					
tra-2^{ts1}/*tra-2*^{ts1}	14	7	3	4	0
tra-2^{ts1}/*CyO*	13	2	0	8	3
wild-type Oregon R	11	1	0	8	2
B					
tra-2^{ts1}/*tra-2*^{ts2}	6	0	0	4	2
tra-2^{ts2}/*tra-2*^{ts2}	8	0	0	3	5
tra-2^{ts2}/*CyO*	10	0	0	9	1

Females mutant for *tra-2*^{ts} were reared at 16°C. Their ovaries were removed within 2 h after eclosion and transplanted into adult wild-type males. These were immediately transferred to 29°C, and the ovaries were scored 4–5 days later in series A, and 10 days later in series B.

blotting. We will refer to the testes and accessory glands as the 'reproductive organs', and to the rest of the fly as the 'carcass'.

No YP were detected in the reproductive organs of the pseudomales under any of the temperature regimes whereas they were consistently present in carcasses in which, as expected from earlier results (Belote *et al.*, 1985; Bownes *et al.*, 1987), the permissive temperature had allowed the YP genes to become active in the cells of the fat body. This difference between the reproductive organs and the carcasses is not due to a higher protein content in the latter as it remains even when 40 reproductive organs and only four carcasses are compared (Figure 2).

To test whether the YP genes were transcribed, but not translated in the reproductive organs of pseudomales, Northern blots were performed. No YP transcripts were observed in the reproductive organs of pseudomales, but they were again found in the carcasses (Figure 3A). A control in which the same filters were probed with cloned DNA of the α_1-tubulin gene indicated that RNA was present in both tracks (Figure 3B).

Thus, the cells of the reproductive organs of *X/X; tra-2*^{ts} pseudomales cannot be stimulated to synthesize YP or to transcribe the YP genes or to take up YP from the haemolymph at 16°C. At this temperature, *tra-2*^{ts} is able to form a functional product, as seen by the initiation of YP synthesis in the fat body of the same flies.

Discussion

In *Drosophila*, the genes encoding the yolk proteins (YP) are subject to sex specific, tissue specific and stage specific regulation. They are active in the fat body and in the follicle cells of adult females. In the present paper, we studied their regulation in the follicle cells which are part of the somatic component of the ovary. These cells stop mitosis and cell division by stage 6 of oogenesis, but transcription and translation continue through the final oogenic stage 14. The first transcripts of the YP genes are detected by stage 8 and start disappearing again by stage 11 (Brennan *et al.*, 1982), indicating a temporal regulation of transcription during the life time of non-dividing cells.

The main point of our results is that the ovaries of *XX; tra-2*^{ts} mutant females can mature and become vitellogenic at the restrictive temperature. We show that YP are synthesized and incorporated into the oocyte in mutant ovaries indicating that the YP genes become active in follicle cells in the absence of a functional product of *tra-2*. This is most convincingly demonstrated in the experiments in which mutant ovaries, before their YP genes are transcribed, were transplanted into male hosts where they became vitellogenic in the absence of any external source of YP.

Without a functional product of *tra-2*, the gonadal anlage of XX animals develops into a testis. An active product of *tra-2*, on the other hand, when present throughout development causes the formation of an ovary with its follicle cells. Once this cell type is formed, its further development to the stages when YP genes become transcriptionally active is independent of *tra-2* function. It thus appears that the regulation of the YP genes has been delegated to a sex specific cell type. This type of regulation was also observed for the genes coding for male specific transcripts (*mst*) in the accessory glands (paragonia) of males (Schäfer, 1986a,b; DiBenedetto *et al.*, 1987). In this tissue, however, the *mst* genes are being transcribed as soon as the corresponding paragonial cells are morphologically recognizable (Chapman

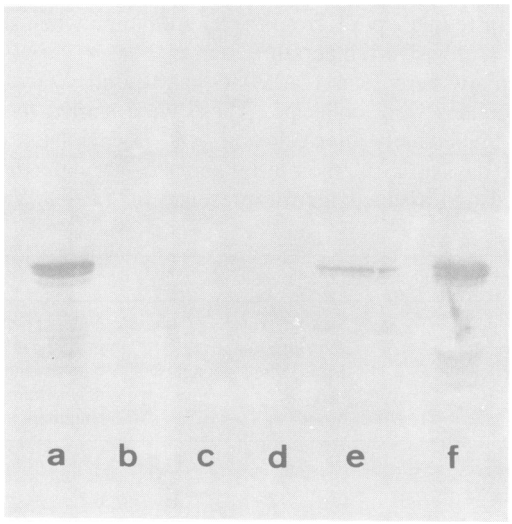

Fig. 2. Synthesis of YP cannot be induced in testes or accessory glands of pseudomales. All animals were reared at the restrictive temperature of 29°C and were transferred to 16°C within 2 h after eclosion. Four days later, the reproductive tissues (testes + accessory glands) and the carcasses were assayed for the presence of YP by Western blotting. (**a**) one Oregon R wild-type female (whole fly); (**b**) one Oregon R wild-type male (whole fly); (**c**) reproductive tissues of 40 Oregon R males; (**d**) reproductive tissues of 40 pseudomales, and (**e**) carcasses for four pseudomales of genotype *X/X; tra-2*^{ts1}/*tra-2*^{ts1}; (**f**) ovaries of one female *X/X; tra-2*^{ts1}/*tra-2*^{ts1}, reared at 16°C.

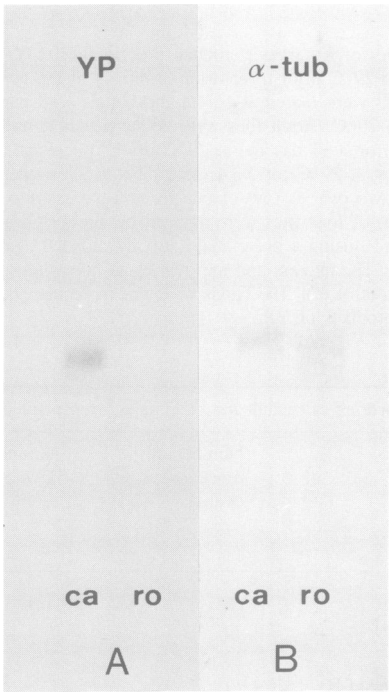

Fig. 3. Transcription of YP genes cannot be induced in reproductive tissues of pseudomales. Animals of genotype *X/X; tra-2*^{ts1}/*tra-2*^{ts1} were reared at 29°C and 0–2 h after eclosion were shifted to 16°C for 4 days. RNA was then extracted from the carcasses (ca) and from the reproductive organs (ro) of 20 pseudomales. Each track was loaded with 5 μg of RNA. **A**, Northern blot probed with *ypl* cloned DNA (entire coding sequence); **B**, Northern blot probed with α_1-tubulin cloned DNA (gift of P.Wensink).

and Wolfner, 1988), whereas the follicle cells must first develop to a specific stage of oogenesis before transcription of the YP genes begins. This further development depends on a cellular interaction with developing female germ cells, as shown by the fact that ovaries with abortive germ cells or without germ cells do not synthesize or take up YP. Since an active product of the *tra-2* gene is required neither for normal oogenesis (Schüpbach, 1982) nor for maturation of the follicle cells (this report), this latter process appears to be independent of *tra-2* function.

The regulation of the YP genes involves *cis* and tissue specific *trans*-acting signals. Wensink and his collaborators (Garabedian *et al.*, 1985, 1986; Logan *et al.*, 1989; Logan and Wensink, 1990) have identified three *cis* regulatory sequences that are necessary for tissue specific expression of the YP genes. Two elements are responsible for expression in the follicle cells. In the fat body cells of adult females, expression of the YP genes depends on an element of 125 bp which acts as an enhancer. We assume that a *trans*-acting factor, specifically synthesized in the fat body cells, must interact with this sequence to allow transcription to take place. In this tissue, the activity of the YP genes is under continuous control of *tra-2* (Belote *et al.*, 1985; Bownes *et al.*, 1987) and, by inference, of the sex specific products of *dsx*, the last member in the regulatory cascade of the sex-determining genes (Baker and Ridge, 1980; Nöthiger *et al.*, 1987; Shirras and Bownes, 1987; Nagoshi *et al.*, 1988). We thus postulated that in the fat body cells, the male specific product of *dsx*, which prevents expression of the YP genes (Bownes and Nöthiger, 1981), may bind to the fat body enhancer element, or to a tissue specific *trans*-acting factor, to block transcription of the YP genes (see Figure 9 in Steinmann-Zwicky *et al.*, 1990). This simplest model accounts for sex, tissue and stage specific expression of YP genes.

As our results indicate, regulation of the YP genes in the follicle cells appears to be different. Here, the role of the sex-determining genes is to direct a group of progenitor cells into the female specific pathway of ovarian development. The differentiation of follicle cells, so we assume, then necessarily leads, at the appropriate stage of oogenesis, to the production of a tissue specific *trans*-acting factor that may recognize and bind to the two DNA sequences identified by Logan *et al.* (1989) as necessary for expression of the YP genes in follicle cells. Our hypothesis predicts that there will be no functionally significant binding of a *dsx* product to these ovarian enhancer sequences.

Our results, together with those of Belote *et al.* (1985), show that the YP genes are subject to different regulatory mechanisms in the follicle cells and in the fat body. This is also reflected in the existence of different DNA sequences conferring expression of the YP genes in the follicle cells on the one hand and in the fat body on the other (Logan *et al.*, 1989; Logan and Wensink, 1990). It is also consistent with the differential hormonal regulation in the two tissues (Bownes *et al.*, 1983a).

Once absence of a functional product of *tra-2* at 29°C has forced the gonadal primordium of *XX; tra-2*^{ts} mutant animals to develop as a testis, re-activation of *tra-2* by shifting the pseudomales to the permissive temperature does not activate the YP genes although it does so in the fat body of the same individuals (Figures 2 and 3). Thus, in contrast to the fat body cells, the postulated *trans*-acting factor is not present in the somatic cells of the testes of *XX; tra-2*^{ts} pseudomales, nor is it inducible in such animals at the permissive temperature. In summary, although gonadal somatic cells of testes and ovaries may be homologous, their sex specific differentiation precludes a flexible and reversible response of the YP genes in *XX; tra-2*^{ts} animals. This again shows regulation by cell type rather than direct control by *tra-2*.

A danger, inherent in all experiments in which a temperature shift has no phenotypic consequence, is that the shift does not, or only to an insufficient degree, alter the structure and activity of the temperature sensitive molecule. In our case, this would mean that the *tra-2*^{ts} protein remains still sufficiently active in the follicle cells after the shift to the restrictive temperature, and sufficiently inactive in the testicular and paragonial cells after the shift to the permissive temperature. In the fat body, on the other hand, shifts in both directions result in corresponding phenotypical changes. These *ad hoc* assumptions are rather unsatisfactory, and they become even more unlikely when one considers that both alleles, *tra-2*^{ts1} and *tra-2*^{ts2}, are fully penetrant in every cell of an XX animal which develops as a female with vitellogenic ovaries at 16°C, and as a male with testes at 29°C. Furthermore, if each follicle cell was making a small amount of YP in response to a residual activity of *tra-2*^{ts}, one would not expect the cells surrounding an oocyte to make sufficient YP to produce a mature egg. This is especially true for ovaries developing in a male host where the fat body produces no YP.

It is formally possible that once *dsx* is expressed in the female specific mode in the follicle cells of *XX; tra-2*^{ts} females, it cannot revert to the male specific mode when such animals are shifted to 29°C. This would mean that the expression of the YP genes in the follicle cells is also under direct control of *dsx*, as it is in the fat body. Sex specific expression of *dsx* which leads to DSX^{F} or DSX^{M} products occurs by alternative splicing (Burtis and Baker, 1989). If the YP genes in the follicle cells were in fact under direct control of *dsx*, the female specific product DSX^{F} would have to be extremely stable since ovaries still contained vitellogenic stages even after 10 days at 29°C (Table III). As demonstrated by the adult fat body, however, it is clear that sexual reprogramming readily occurs, even in this tissue which consists of stationary polyploid cells (Johnson and Butterworth, 1985; Bownes and Reid, 1990). This implies that the sex-determining genes, at least *dsx*, are transcriptionally active in non-dividing cells, and that sex specific splicing of the *dsx* transcripts does occur. This supports our conclusion that the YP genes in the gonadal soma are regulated by cell type.

Materials and methods

Fly stocks

Fly stocks were maintained on standard food (yeast, cornmeal, sugar and agar) at 25°C. Wild-type flies were of the Oregon R strain. Two *tra-2*^{ts} alleles were used (Belote and Baker, 1982, 1983), kept in balanced stocks: *B*^{S}*Y; tra-2*^{ts1} *bw/CyO* and *B*^{S}*Y; tra-2*^{ts2}*bw/CyO*. For gene symbols see Lindsley and Grell, 1968; Lindsley and Zimm, 1985, 1990.

The temperature shifts with adult flies were done in preconditioned food vials.

Transplantation experiments

Larval ovarian anlagen or immature adult ovaries were transplanted according to the technique developed by Ephrussi and Beadle (1936), described by Ursprung (1967). The stocks and crosses used to generate the larval donors and hosts for the transplantation of ovarian anlagen are given in the footnote

to Table I. The dominant mutation ovo^{D1} blocks oogenesis and renders females sterile with small and undeveloped ovaries.

Observation of oogenesis

Ovaries were dissected in Ringers solution (Chan and Gehring, 1971) and subsequently mounted under a coverslip for inspection with phase-contrast microscopy. Stages of oogenesis are given according to King (1970).

Analysis of yolk proteins

Flies were dissected and divided into reproductive organs (ovaries or testes with accessory glands) and carcass (rest of the fly). The proteins were solubilized in 50 μl Laemmli buffer (Laemmli, 1970) and the yolk proteins (YP) were analysed by polyacrylamide gel electrophoresis (Bownes *et al.*, 1983a) and by Western blotting. The polyclonal antibody was raised against YP1, YP2 and YP3 cut from SDS polyacrylamide gels (Kozma and Bownes, 1986).

Northern blots

Isolation of RNA. RNA was isolated from reproductive organs, carcasses and whole flies as described by Bownes *et al.* (1983b). Northern blots were made onto Hybond membrane (Amersham) as described by Thomas (1980) and modified by Bownes *et al.* (1983a).

Preparation of ^{32}P-labelled probes. The whole *ypl* gene, or α_1-tubulin DNA, was cloned into a pGem vector. 25 ng of either pGemYP1 or pGemα_1 was labelled by random priming to a specific activity of $\sim 10^9$ counts/min/μg (Feinberg and Vogelstein, 1983).

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