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Monitoring positional information during oogenesis in adult *Drosophila*

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Summary

About 184 P[lac,ry$^+$]A insertions (O'Kane & Gehring, 1987) have been incorporated into the genome *via* P element-mediated transformation. The temporal–spatial localization of β-galactosidase, synthesized by these insertions during oogenesis, is described. 32 % present control levels of endogenous β-galactosidase expression and 68 % show novel patterns. 13 % of the insertions are germline-specific; 33 %, follicle-cell-specific; 20 % are expressed in both germ line and follicle cells; and 2 %, specific to the germarium. Several lines exhibit strict temporal–spatial localizations of β-galactosidase; notably those expressed in specific populations of follicle cells. The results are discussed with respect to some of the positional information encoded in the genome to which the insertions respond, the use of the insertions as markers for cell differentiation and the potential of the technique for isolating new genes involved in egg production.

Key words: *Drosophila*, transformation, β-galactosidase, P[lac,ry$^+$]A, oogenesis.

Introduction

Embryos result, in part, from genetic and cellular interactions occurring in a temporally and spatially precise manner during oogenesis. In order to understand oogenesis, it is essential that the genetic and molecular components of the process are known. In *Drosophila melanogaster*, genetic and molecular elements have been isolated which are required for normal polarity of the egg (Bull, 1966; Nüsslein-Volhard *et al.* 1980; Lohs-Schardin, 1982; Frohnhöfer & Nüsslein-Volhard, 1986; Lehmann & Nüsslein-Volhard, 1986; Schüpbach & Wieschaus, 1986*a*; Degelmann *et al.* 1986; Anderson, 1987), vitellogenesis (Brennen *et al.* 1982), egg shell production (Digan *et al.* 1979; Spradling & Mahowald, 1980; Komitopoulou *et al.* 1983; Burke *et al.* 1987) or general fertility (Gans *et al.* 1975; Mohler, 1977; Perrimon *et al.* 1986).

Two basic cell types can be distinguished during oogenesis (King, 1970): the germ cells, consisting of fifteen nurse cells and an oocyte, and surrounding these, the somatically derived follicle cells. Many maternal genes have been tested for somatic-, or germline-, dependent functions, using pole cell (e.g. Wieschaus *et al.* 1978), or ovary (Clancey & Beadle, 1937), transplantation experiments or mitotic recombination (Wieschaus *et al.* 1981; Perrimon & Gans, 1983). For the genes required uniquely during oogenesis, both germline- (Wieschaus *et al.* 1978; Schüpbach & Wieschaus, 1986*b*) and somatic-line- (Perrimon & Gans, 1983; Schüpbach, 1987) dependent gene activity has been reported. In one case, the gene dicephalic is required in both germline and somatic cells (Frey & Gutzeit, 1986).

Precise knowledge of the positional information elaborated during oogenesis exists for those genes where spatial distributions of messages, or proteins, have been examined. Localization of transcripts from the dorsal (Steward *et al.* 1985), bicoid (Frigerio *et al.* 1986), caudal (Mlodzik *et al.* 1985) and *fs(1)K10* (Haenlin *et al.* 1987) genes have been described. Caudal, dorsal and bicoid transcripts are made by the nurse cells and shunted into the oocyte chamber. Bicoid is different from the others, in that its transcripts are localized to the anterior part of the oocyte chamber; the others exhibit even distributions within the oocyte. Messages from the *fs(1)K10* gene surround the oocyte nucleus only.

Message sequences unique to the follicle cells include those coding for proteins of the vitelline membrane (Burke *et al.* 1987) and the chorion (Parks & Spradling, 1987). Generally, these mark all the follicle cell populations at specific times during oogenesis although three transcripts that are thought to code for minor chorion proteins are localized to

specific groups of follicle cells.

A new technique, described by O'Kane & Gehring (1987), can be applied to probe the problem of the positional cues existing during oogenesis. Briefly, position- and temporal- specific regulatory elements can be revealed, by inserting at random into the genome a ubiquitously acting promotor linked to the structural gene for β-galactosidase. This enzyme can then be monitored, in the individual lines obtained, to examine the positional information existing in the different cell types, to which these regulatory elements respond.

Here we report the expression of at least 184 P[lac,-ry$^+$]A (O'Kane & Gehring, 1987) insertions during oogenesis, introduced into the germline *via* P element-mediated transformation (Rubin & Spradling, 1982). The results are discussed with respect to some of the different types of positional information evolving during oogenesis and the potential of the technique for isolating new genes involved in egg production.

Materials and methods

Injection and transformation

150 μg ml^{-1} of pLacA92 (O'Kane & Gehring, 1987) and 50 μg ml^{-1} of pπ 25.7 wc (Karess & Rubin, 1984) plasmid DNA were coinjected into the posterior pole of 0–1·5 h old ry^{506} embryos, as described previously (Rubin & Spradling, 1982; O'Kane & Gehring, 1987). Surviving G_0 adults were crossed separately to ry^{506} flies of the relevant sex. Transformants were distinguished by the presence of ry^+ flies in the following generation (G_1). Between one and five ry^+ individuals, deriving from each G_0 parent, were then crossed individually to ry^{506} flies to amplify the transformed lines.

β-galactosidase activity

ry^{506} controls, in addition to five to ten ry^+ adult females from each line, were selected in the next, or following, generation, to test for β-galactosidase activity. Abdomens were removed from the heads and thoraces and the ovaries squeezed through the cut part of the abdomen. Abdomen and ovaries were fixed in 2·5% glutaraldehyde in 50 mM-Pipes pH 7·5, for 10 min (modified from Lis *et al.* 1983), washed in phosphate buffer solution (PBS), then submerged in 0·2% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) in 5 mM-$K_4Fe(CN)_6$, 5 mM-$K_3Fe(CN)_6$ in PBS overnight at 37°C (as described by Hiromi *et al.* 1985 and O'Kane & Gehring, 1987). Ovaries were then mounted, after separation of ovarioles, in 90% glycerol in PBS, and scored using the compound microscope.

Chromosomal localization

Several inserts showing position-specific patterns were localized using classical genetic crosses to balancer chromosomes. Single ry^+ males were crossed to $CyO/T(2;3)ap^{Xa}/TM3,Sb\,ry^{RK}$ females (see Lindsley & Grell, 1968 for genetic terminology). Where possible, single *Cy Sb* ry^+ males were put with virgin ry^{506} females. In the resulting progeny, ry^+ segregating from *Cy* shows that the insert(s) is (are) localized on chromosome 2. Inserts on chromosome 3 are revealed if ry^+ segregates from *Sb*. Absence of ry^+ males in F_1 localizes inserts to the X chromosome. Following their chromosomal localization, individual inserts were balanced with relevant balancer chromosomes. These were retested for β-galactosidase activity, substituting a 30 min fixation in 4% paraformaldehyde for glutaraldehyde, which gave improved morphology.

Some of the insertions have been localized by *in situ* hybridization to polytene chromosomes of salivary glands. P[lac,ry$^+$]A plasmid DNA was labelled with biotinylated 11-dUTP using random primer extension (Feinberg & Vogelstein, 1983). The probe was then hybridized to squashes of certain transformed lines. Conditions of hybridization, post-hybridization washes and detection were done as described by Engels *et al.* (1986).

Results

Transformation and number of insertions

We found 128 G_1 transformants from 1928 injected eggs. Since more than one insert can be incorporated into the germline of a single injected egg, this number is the minimum number of inserts tested. Following the analysis of β-galactosidase activity in the oocytes of different lines, deriving from single G_0 flies, different patterns were encountered in 51 cases. This shows that at least two insertion events have occurred in these cases and raises the number of inserts to at least 179. In addition, a single germline cell may attain two or more insertions. If these segregate, two or more different patterns will be observed. Only five lines gave two different patterns of β-galactosidase activity in the five to ten flies tested. This means that at least 184 inserts have been tested here. In the present study, linked insertions will not have been noticed except where hybridization *in situ* to polytene chromosomes has been performed. So far, *in situ* hybridization of P[lac,ry$^+$]A to the polytene chromosomes of 38 lines has been performed; two lines proved to have two linked insertions and the rest only one.

Expression of P[lac,ry$^+$]A insertions

The adult ovary is made up of several cell types (King, 1970; Mahowald & Kambysellis, 1980; Margaritis *et al.* 1980 and Fig. 1). A cluster of parallel ovarioles, enveloped in both a muscular peritoneal, and an epithelial, sheath makes up each ovary. We use the nomenclature of King (1970), who has divided oogenesis into 14 stages starting in the germarium and ending with mature stage-14 oocytes. Cells making up the ovary are of two distinct origins: first, the germline, giving rise to the oocyte and nurse cells, derives

from the pole cells during embryogenesis; second, the somatic follicle cells that derive from certain cells of the mesoderm during embryogenesis. Control ry^{506} ovaries possess endogenous β-galactosidase activity when glutaraldehyde is used as a fixative. This is limited to tissue attached to the posterior end of stage-14 oocytes and variably, but always weakly, at the base of the chorionic appendages. No endogenous β-galactosidase activity was observed when paraformaldehyde was used. The β-galactosidase activities of the P[lac,ry^+]A insertions have been grouped with respect to cell type: 59 (32%) exhibited no β-galactosidase activity significantly different from controls; 3 (2%) were specific to the germarium; 23 (13%) expressed β-galactosidase only in germline; 61 (33%) were specific to the follicle cells; and 36 (20%) were expressed in both follicle and germline cells. Three lines expressed β-galactosidase in cells of the peritoneal, or epithelial, sheaths; only one of which was specific to these cells.

In the following sections, the characteristics of the germline- and follicle-cell- specific lines are described. Lines expressing β-galactosidase in both germline and follicle cells will not be discussed further since β-galactosidase was distributed evenly in all cells.

Germline-specific insertions

In the germarium, the germline consists of about two to three stem cells per ovariole (Wieschaus & Szabad, 1979), which divide to give one pro-oocyte leaving a stem cell in place. The pro-oocyte divides four times to give sixteen cells; one of these becomes the oocyte and the other fifteen give rise to the nurse cells (King, 1970 and Fig. 1). During stages 1–9, the nurse cell nuclei polytenize, being localized in the anterior part of each follicle.

Of the 184 insertions, 23 (13%) expressed β-galactosidase specifically in the cells of the germline. Ten were expressed within the germarium and all subsequent stages of oogenesis. Three initially expressed β-galactosidase during stages 1–6, and for ten lines β-galactosidase activity was detected initially during stages 8–10. Two lines were restricted to stage

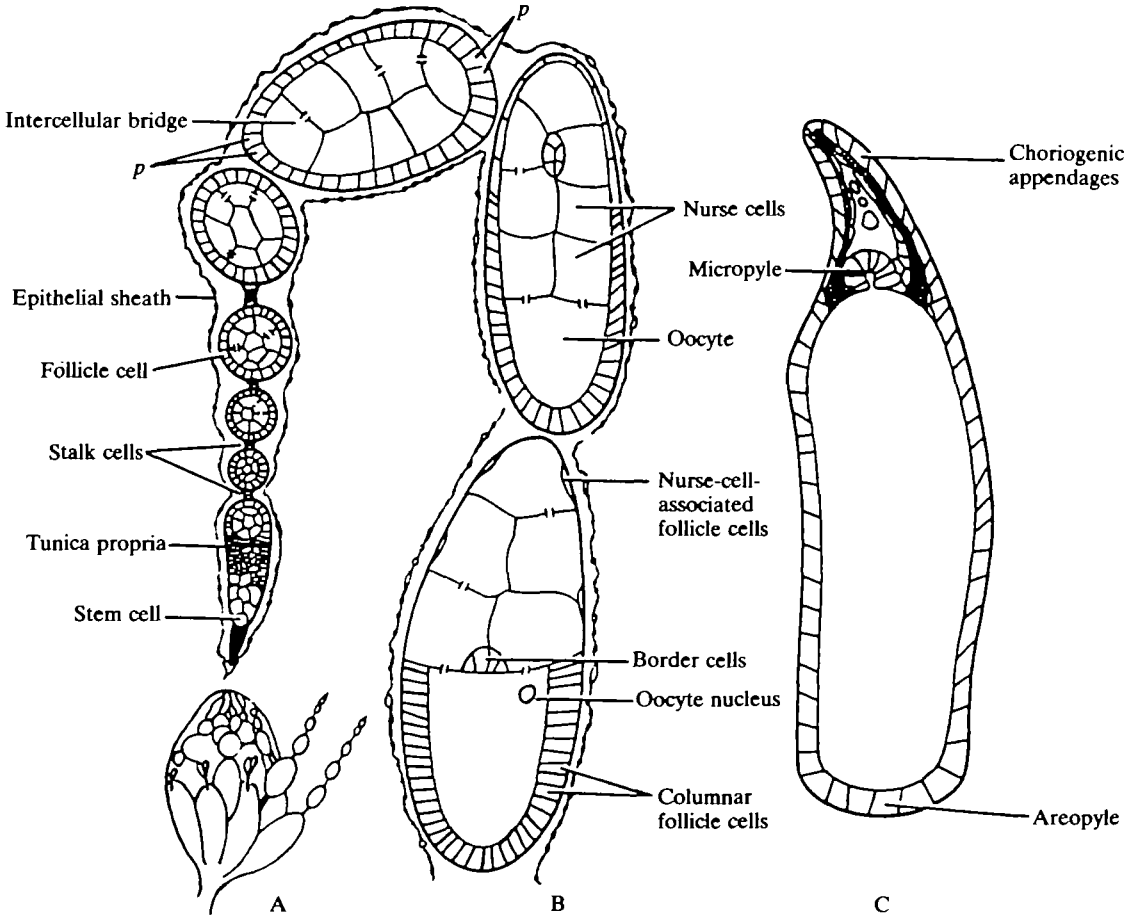


Fig. 1. Diagram of the adult ovary (after King, 1970). (A) 10–20 ovarioles are packed into each ovary. Two ovarioles are pulled away from the ovary to reveal the individual follicles. (B) A single ovariole with numbers denoting the various stages of oogenesis. The polar cells (*p*) are indicated on the stage-7 follicle at the top of the diagram. (C) A stage-13 follicle.

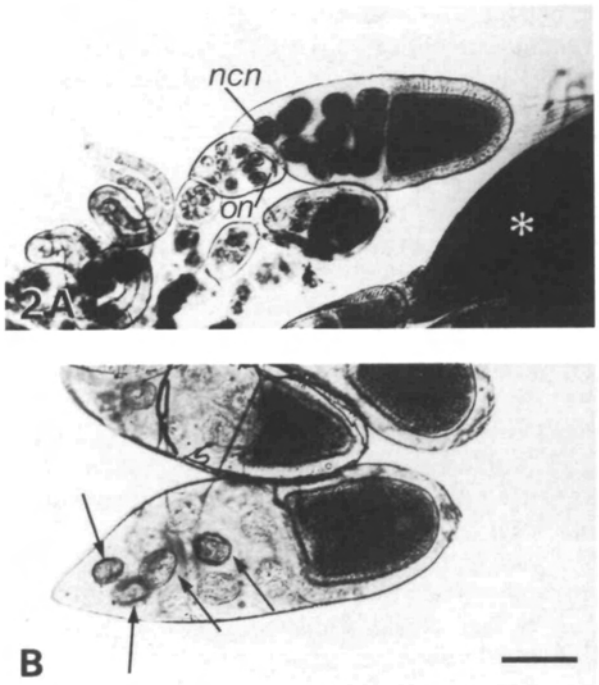


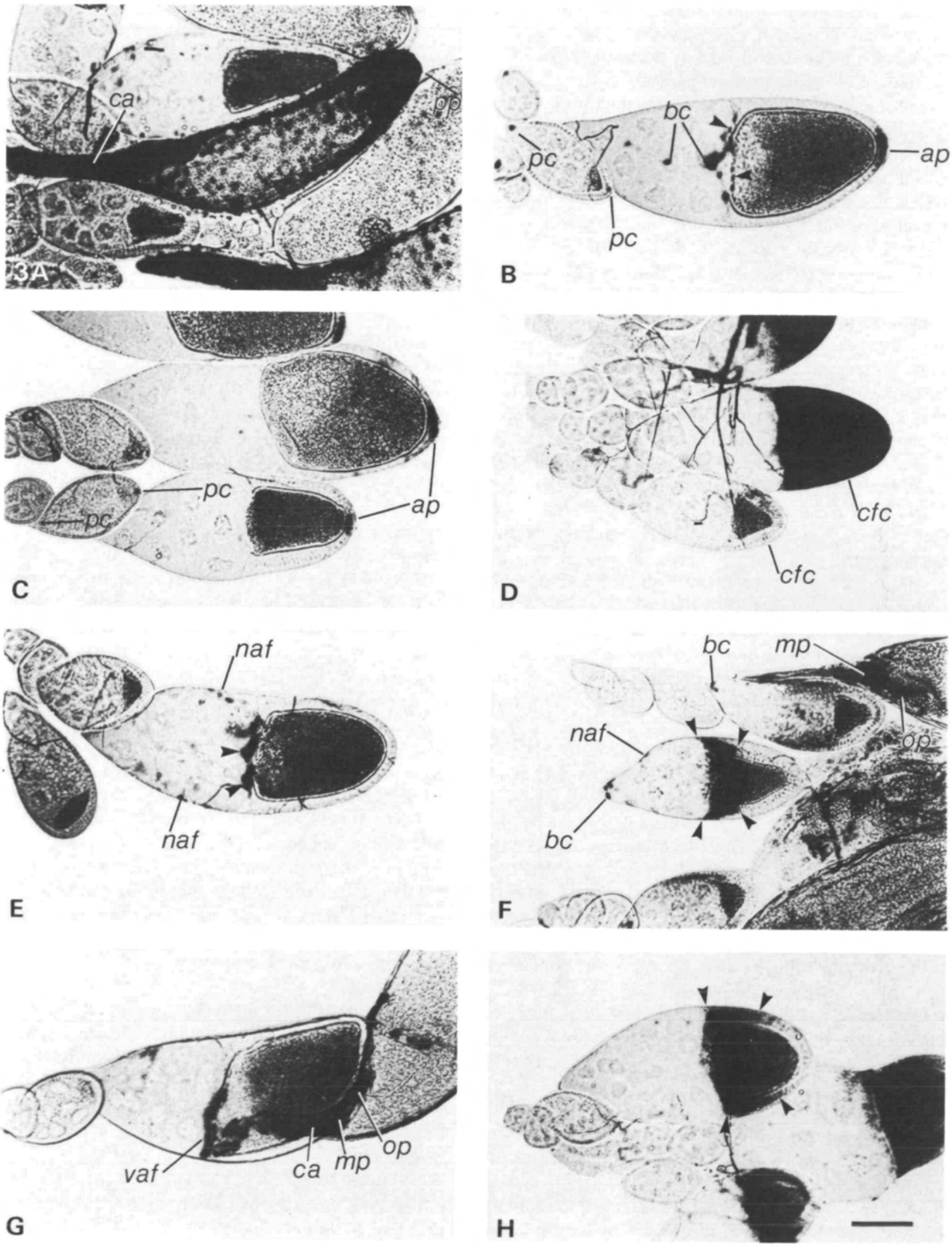
Fig. 2. Two examples of P[lac,ry$^+$]A insertions exhibiting β-galactosidase activity in the germline. (A) M34a, localized at 70D-F, presents the blue precipitate around the nurse cell nuclei (*ncn*), detectable throughout oogenesis, and the oocyte (*on*) nuclei, initially detectable at stage 7. Stage-14 oocytes (*) are strongly marked. (B) Two stage-10 oocytes from line P6a, localized at 38A-B; of the 15 nurse cells, four nuclei, localized in the anterior portion of the nurse cell chamber, possess strong β-galactosidase activity (arrows). Three others consistently present β-galactosidase activity (weakly marked in this preparation). The rest show weak staining. No β-galactosidase activity is detectable over the oocyte nucleus. Bar, 100 μm.

10 and labelled the nurse cell nuclei only. Six lines exhibited simultaneous expression over nurse cell and oocyte nuclei (Fig. 2A). In one case, P6a, five to eight of the fifteen nurse cell nuclei consistently gave stronger β-galactosidase activity; the remaining nurse cells were weakly labelled (Fig. 2B). Other spatial differences, such as gradients (Mlodzik *et al.* 1985), localized expression in the oocyte (Frigerio *et al.* 1986; Haenlin *et al.* 1987; Aït-Ahmed *et al.* 1987) or β-galactosidase activity occurring strictly in specific nurse cells, were not encountered.

Follicle-cell-specific insertions

In the germarium, about 80 cells of mesodermal origin surround each cluster of sixteen germline cells. By successive mitoses, these 80 cells give rise to approximately 1200 cells per follicle upon completion of stage 6. During stages 1–6, two morphologically different follicle cell types can be seen (King, 1970); those surrounding the sixteen germline cells and six to ten stalk cells joining each follicle (King, 1970). Throughout stages 8 and 9, the bulk of the follicle cells start to migrate posteriorly over the oocyte chamber. By stage 10, 50–100 squamous cells are left covering the nurse cells whereas about 900 columnar cells surround the oocyte chamber (Margaritis *et al.* 1980). A group of six to ten border cells migrate from the anterior tip of the follicle, through the nurse cell complex to the anterior part of the oocyte chamber, during stages 8–10. These then migrate dorsally to lie opposite the oocyte nucleus. Migration of about 300

Fig. 3. Temporal and positional expression of eight P[lac,ry$^+$]A insertions in different populations of follicle cells. (A) R8a, localized at 58A-D, expresses β-galactosidase only during stage 13 and 14 of oogenesis; all nuclei, especially in the choriogenic appendages (*ca*) and at the posterior pole (*pp*), possess enzyme activity. (B) Q21a, localized at 36B-C, exhibits β-galactosidase activity in two anterior and two posterior polar cells (*pc*) during stages 3–8. After migration of the border cells (*bc*), which are blue during stage 10, a population of 30–40 cells between the nurse cells and oocyte chamber stain intensely (arrowheads). A single blue border cell has been left trapped between the nurse cells in this stage-10 follicle. In addition, a set of 15–20 cells at the posterior pole, or the future areopyle (*ap*) are marked. (C) R1b, localized at 70A, is initially much like Q21a labelling 2 or 3 polar cells (*pc*). During stage 10, however, only 12–15 cells at the posterior pole (*ap*) are marked. (D) S17b, localized at 85F, is weakly expressed during stage 9 (lower *cfc*), then shows intense β-galactosidase activity during stage 10 in the columnar follicle cells covering the oocyte (upper *cfc*). (E) L53b, localized at 61F-62A, is initially active during stage 9. Only the nuclei of the 30–40 nurse-cell-associated follicle cells (*naf*) plus 7–10 cells lying between the oocyte chamber and nurse cell complex are labelled (arrowheads). By stage 14, cells surrounding the micropyle, in addition to isolated cells of the operculum, stain (not shown). (F) K59a, localized at 57B, is active initially during stage 6. In the stage-8 oocyte shown, a group of migrating follicle cells (between arrowheads), the nurse-cell-associated follicle cells (*naf*) and the population of cells giving rise to the border cells (*bc*) are marked. By stage 14, cells around the micropyle (*mp*) and operculum (*op*) label strongly. (G) R15b: two linked inserts localized at 90F-91A and 95C were found. Following separation of the insertions by recombination it was shown that the former one gave the observed β-galactosidase pattern. β-galactosidase activity is seen surrounding the nuclei of 20–30 ventroanterior cells (*vaf*) during stage 10.90–100 nuclei labelled during stage 14 are specific to the choriogenic appendages (*ca*), plus cells around the micropyle (*mp*) and the operculum (*op*). (H) M8a, localized at 25B-C; a group of anterior follicle cells covering the oocyte chamber possess β-galactosidase activity (between arrowheads). Follicles shown are early stage 10. All photographs are at the same magnification; bar, 100 μm. All preparations were fixed with 4% paraformaldehyde (see Materials and methods).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follicle cells, between the nurse cell complex and oocyte chamber, occurs during stage 10. These are destined to make the respiratory appendages and the operculum (see Margaritis *et al.* 1980).

A total of 61 inserts gave β-galactosidase expression specific to the follicle cells. 27 were expressed in all follicle cells and 34 in specific sets of follicle cells. Of the 27 lines, fourteen coloured the follicle cells throughout oogenesis, nine colouring initially around stage 8–10, one specific to stage 10, and three specific to stages 13 and 14 (Fig. 3A).

Of the 34 lines showing position-specific localization of β-galactosidase in the follicle cells, 17 were specific to the border cells. The majority of lines in this class exhibited enzyme activity during stages 8–14, allowing the migration of the border cells to be followed. At the anterior tip of the follicle, six to ten cells were marked in stage-10 oocytes. For all 17 lines, β-galactosidase was concentrated around the micropyle in stage-14 oocytes, which is consistent with the idea that one of the functions of border cells is to fabricate this structure (King, 1970). White *et al.* (1984) have described an antigen specific to the border cells.

A group of nine lines showed labelled cells at both poles of the follicles (Fig. 3B and C); five were coloured initially around stage 3, two during stage 6, and two during stages 8–9. All of these lines exhibited β-galactosidase activity in the border cells during stages 8–14. These patterns suggest a common feature at the anterior and posterior poles of the follicles, with respect to gene activity. One line in this class labels two or three polar cells (see Brower *et al.* 1981) during stages 6–8. Later during stages 10–14, a group of twelve to fifteen cells are left labelled at the posterior pole (Fig. 3C). These correspond to the central pole cells or areopyle described by Margaritis *et al.* (1980).

The eight lines remaining also mark unique sets of follicle cells. S17b labels only the columnar follicle cells covering the oocyte chamber at stage 10 (Fig. 3D). Two lines, K59a and L53b, mark similar sets of follicle cells; one set consisting of a group of cells migrating between the nurse cells and oocyte chamber, and the other, the 50–100 squamous cells surrounding the nurse cells (Fig. 3E and F). K59a differs from L53b in that β-galactosidase activity starts during stage 8 in the cells migrating over the nurse cell chamber (Fig. 3F). Initial β-galactosidase expression, specific to L53b, begins during stage 10 (Fig. 3E). Fig. 3G shows the β-galactosidase activity of R15b: in late stage-10 oocytes, a group of 20–30 ventral follicle cells migrating between the nurse cells and the oocyte are coloured; by the end of stage 10, some dorsal cells become active for β-galactosidase; and, during stage 14, β-galactosidase activity is detected over the 90 or so nuclei surrounding each choriogenic appendage (Fig. 3G) and in some cells in the operculum. Line Q26b labels the stalk cells and adjacent follicle cells in early (stages 2–6) follicles. During stage 8, all columnar follicle cells are marked; later, labelling of posterior ventral cells disappears (not shown). Lines M8a (Fig. 3H) and M32b label a large subset of the anterior columnar follicle cells. Both lines label stage-10 oocytes specifically, M8a labelling fewer cells than M32b at this stage.

Discussion

The expression of at least 184 P[lac,ry$^+$]A insertions has been examined for β-galactosidase activity during oogenesis. About a third present no β-galactosidase activity in any cell making up the ovary. The rest express β-galactosidase in the follicle cells (33 %), the germline (13 %), or both the germline and the follicle cells (20 %).

A set of unique temporal and spatial patterns of enzyme activity are shown for the follicle cell populations (Fig. 3 and Table 1). Since this technique reveals the existence of different regulatory elements (O'Kane & Gehring, 1987), the results show the potential for the positional information elaborated in the genome during oogenesis, to which the insertions respond. On the basis of morphology, Margaritis *et al.* (1980) have described ten different groups of follicle cells. The spatial localization of β-galactosidase, fabricated by the insertions, correspond to these cell types: enzyme activity has been found within the columnar cells (Fig. 3D); the central cells at the posterior pole (Fig. 3B and C); the cells of the dorsal appendages (Fig. 3E); the operculum (Fig. 3F); the border cells (Fig. 3B); and the nurse-cell-associated follicle cells (Fig. 3E). Positional cues, as revealed here, are not only restricted to cell type; lines M8a (Fig. 3H) and M32b are expressed in different subsets of columnar follicle cells during stage 10.

Different sets of follicle cells migrate between the nurse cells and oocyte chamber during stage 10 (Margaritis *et al.* 1980). In the present study, a more precise idea of the origin and migration of these follicle cell populations, with respect to gene activity, has been established. R15b (Fig. 3G) labels initially 20–30 ventral cells; Q21a (Fig. 3B) marks 30–40 dorsolateral cells; Q26b labels about 200 anterior, and particularly dorsal, cells; L53b (Fig. 3E) is expressed in 20–30 dorsolateral cells; and K59a (Fig. 3F) marks approximately 100 dorsoanterior cells, during stage 10. In addition, as oogenesis evolves, an increase in the number of cells expressing β-galactosidase, has been observed; R15b, for example, labels 20–30 cells

Table 1. *Summary of β-galactosidase expression and cytological localization of some P[lac,ry$^+$]A insertions*

Line	Cytological localization	Temporospatial description of β-galactosidase
M34a	70D-E	Germ line cells throughout oogenesis. Fig. 2A.
P6a	38A-B	Stage 10: nurse cells, anteriorly located more intense than the rest. Stage 14: oocyte. Fig. 2B.
R8a	58A-D	Stage 13–14: all follicle cells. Fig. 3A.
R1b	70A	Stage 6–8: polar cells. Stage 10–14: 12–15 cells of areopyle. Fig. 3C.
Q21a	36B-C	Stage 6–8: polar cells. Stage 10: border cells, cells of areopyle plus 30–40 dorsolateral cells between nurse cells and oocyte. Stage 14: follicle cells around micropyle and some in the operculum. Fig. 3B.
S17b	85F	Stage 8–10: columnar follicle cells. Stage 14: all follicle cells. Fig. 3D.
K59a	57B	Stage 8: 150–200 follicle cells migrating over the oocyte, nurse cell associated follicle cells. Stage 10: 100–150 dorsoanterior, including 50–100 nurse cell associated follicle cells. Stage 14: follicle cells of operculum, micropyle and several in appendages. Fig. 3F.
L53b	61F-62A	Stage 10: 50–100 nurse cell-associated follicle cells and 20–30 dorsolateral cells between nurse cells and oocyte. Stage 14: 20–30 cells at base of micropyle and 5–10 scattered in the operculum. Fig. 3E.
R15b	90F-91A	Stage 10: 20–30 ventral cells, with progressively more dorsal cells. Stage 14: dorsal appendages and base of the micropyle. Fig. 3G.
Q26b	56F1-4	Stage 3–6: stalk cells and adjacent follicle cells. Stage 8–9: all columnar follicle cells. Stage 10: loss of enzyme activity from posterior pole. Stage 14: dorsal appendages, operculum and scattered cells in dorsoanterior part of main body.
M32b	61D-E	Stage 10: anterior columnar follicle cells.
M8a	25B-C	Stage 10: fewer anterior columnar follicle cells. Fig. 3H.

during stage 10, and later, during stage 14, about 200 cells are labelled in the dorsal appendages. A similar spreading effect of gene activity has been observed for the X-linked, chorion gene cluster (Parks & Spradling, 1987), which is initially expressed in a small group of anterior dorsal cells and later, in all, or the majority, of the follicle cells.

The inherent polarity of the follicles with respect to gene activity is demonstrated (Fig. 3). R1b labels only cells at the posterior pole; Q21a marks these cells and a group of anterior cells. Others such as K59a, L53b and M8a specifically mark sets of anterior cells. Brower *et al.* (1981) have described the spatial localization of an antigen which labels predominantly the polar cells of the follicles, in a fashion similar to line Q21a. The potential for gene activity in the oocyte, or in either one or both poles of the embryo is suggested by the existence of certain germline-dependent mutations. These affect the development and differentiation of anterior, posterior, or both, poles of the embryo (Schüpbach & Wieschaus, 1986*a*; Fröhnhofer & Nüsslein-Volhard, 1986), or the oocyte and the embryo (Mohler & Wieschaus, 1986; Frey & Gutzeit, 1986). Our observations show that, with respect to gene activity, the follicle cells also possess common and unique types of positional information at the poles.

Recently, it has been shown that the follicle cells and germline interact during oogenesis for normal egg production (Schüpbach, 1987; Parks & Spradling, 1987). The germline-dependent mutations *fs(1)K10* and gurken (Wieschaus *et al.* 1978; Schüpbach, 1987) alter the morphology of the somatically derived egg shell, in addition to the embryo. Conversely, the somatic-cell-dependent mutation torpedo changes the dorsal part of the embryo, in addition to the egg shell (Schüpbach, 1987). The P[lac,ry$^+$]A insertions described could be combined with these and other maternal effect mutations (e.g. Wieschaus *et al.* 1981) to test whether the regulatory sequences identified depend on the wild-type function of these genes for their expression.

Fewer germline-specific P[lac,ry$^+$]A insertions were found compared to insertions marking the follicle cells (13 % *versus* 32 %). The majority of these expressed β-galactosidase in the fifteen nurse cells and then injected the protein into the oocyte chamber, mimicking the process described for certain proteins (White *et al.* 1984; Gratecos *et al.* 1987) and transcripts (Steward *et al.* 1985; Mlodzik *et al.* 1985). Six of the twenty-four germline insertions express β-galactosidase in the nurse cells and over the oocyte nucleus (Fig. 2A). Blue precipitate was found around the germinal vesicle, as early as stage 6 (Fig. 2A). Assuming that presence of β-galactosidase over the germinal vesicle indicates synthesis at, and not transport to, this site, then this suggests that transcription and translation from the oocyte nucleus may occur as early as this stage of oogenesis. A similar observation for transcription was noted by Haenlin *et al.* (1987) and Aït-Ahmed *et al.* (1987). None of the lines possessed β-galactosidase activity specific to the oocyte nucleus, as described for *fs(1)K10* (Haenlin *et al.* 1987). None marked specific nurse cells, though

two lines labelled certain nurse cells more intensely than others (Fig. 2B).

The technique of O'Kane & Gehring (1987) may lead to the isolation of new genes involved in egg production. For the insertions described (Figs 2, 3), all are viable as homozygotes and show no evident maternal phenotype. This indicates that the majority of the insertions preferentially insert in regions of the genome nonessential to normal gene activity. An essential question therefore is raised: do genes exist in the neighbourhood of the insertions with spatiotemporal patterns of expression similar, or identical, to any particular β-galactosidase pattern? As discussed by O'Kane & Gehring (1987), molecular and genetic approaches should resolve this question. In the absence of such experiments we can only speculate as to the site specificity of the insertions. For example, none of the germline-specific lines exhibit β-galactosidase activity restricted to parts of the oocyte chamber, as is described for bicoid (Frigerio *et al.* 1986), *fs(1)K10* (Haenlin *et al.* 1987) or a transcript from the *yema* region (Aït-Ahmed *et al.* 1987). Either we have not examined enough insertions or β-galactosidase does not lend itself to local expression within the oocyte. At present nothing is known as to the mechanisms involved for the localization of the transcripts within the oocyte chamber. If some quality of the message (e.g. its sequence) is necessary for its localization, presumably β-galactosidase protein will not reflect the spatial localization of the neighbouring transcript. Similarly, genes giving products with subcellular localizations within the nucleus or mitochondria, for example, or products that are transported, will not be picked up for similar reasons. Despite these potential pitfalls, it is noteworthy that β-galactosidase under the control of known regulatory sequences reflects fairly closely the expression of the products, normally under their control, in the cellular system of the embryo (Hiromi *et al.* 1985). Thus we hope that some of the patterns, particularly in the follicle cells, will reflect the spatiotemporal expression of a gene, closely linked to the corresponding insertion, unless many archaic or nonfunctional regulatory sequences exist in the eukaryotic genome.

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