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DEVELOPMENTAL GENETICS OF OOGENESIS

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I. INTRODUCTION

Oogenesis encompasses some of the most fascinating biological processes. Patterning and constructing a new adult multicellular animal begin at the time of oocyte determination. The relatively simple early steps offer both a logical and practical place to focus efforts at understanding developmental programming. Egg cells eventually undergo extraordinary modifications enroute to fertilization. The cell cycle is shunted into meiosis, chromosomes are reorganized, and cell size and nucleocytoplasmic ratios are distorted, due to a host of preparatory activities for embryogenesis. The possession of both germ-line and somatic cells also sets the gonads apart from other tissues. Many organisms segregate germ-line cells away from somatic cells very early in development, where they escape modification, and independently regulate fundamental processes such as sex determination. Nevertheless, in the ovary, germ-line cells must interact extensively with their somatic neighbors during all stages of egg development. Clearly, the attraction biologists with diverse interests have for the process of oogenesis is easy to understand.

Interest in *Drosophila* oogenesis has recently been growing. Mechanisms regulating embryonic segmentation have been traced back to events that structure the egg during oogenesis (Manseau and Schüpbach 1989a; Nüsslein-Volhard and Roth 1989). Fundamental processes of cell biology are becoming accessible by studying the specialized and exaggerated forms found in the egg. Although several excellent general reviews are available (see King 1970; Mahowald and Kambysellis 1980), they cover literature only through 1979. This chapter attempts to summarize the large body of developmental genetic studies carried out since 1980. In addition, it is hoped that some of the speculative ideas discussed will provoke investigation of the many areas where knowledge remains extremely limited.

Our understanding of embryonic segmentation has advanced dramatically by applying a particular developmental genetic strategy. Most of the genes involved in specific developmental pathways were defined genetically in large mutant screens that approached saturation. Gene position and function within hierarchies were revealed by genetic and developmental experiments, followed by molecular analysis. This chapter begins by examining the current status and difficulties associated with applying such an approach to oogenesis. Subsequently, current knowledge of the steady-state process of oogenesis in the adult gonad is summarized. Information on preadult development of the ovary and on most aspects of ovarian physiology can be found in previous reviews (King 1970; Mahowald and Kambysellis 1980). Although dorsal-ventral and anterior-posterior patterning of the embryo begins during oogenesis, these topics are not treated in detail since separate chapters in this volume are devoted to these subjects.

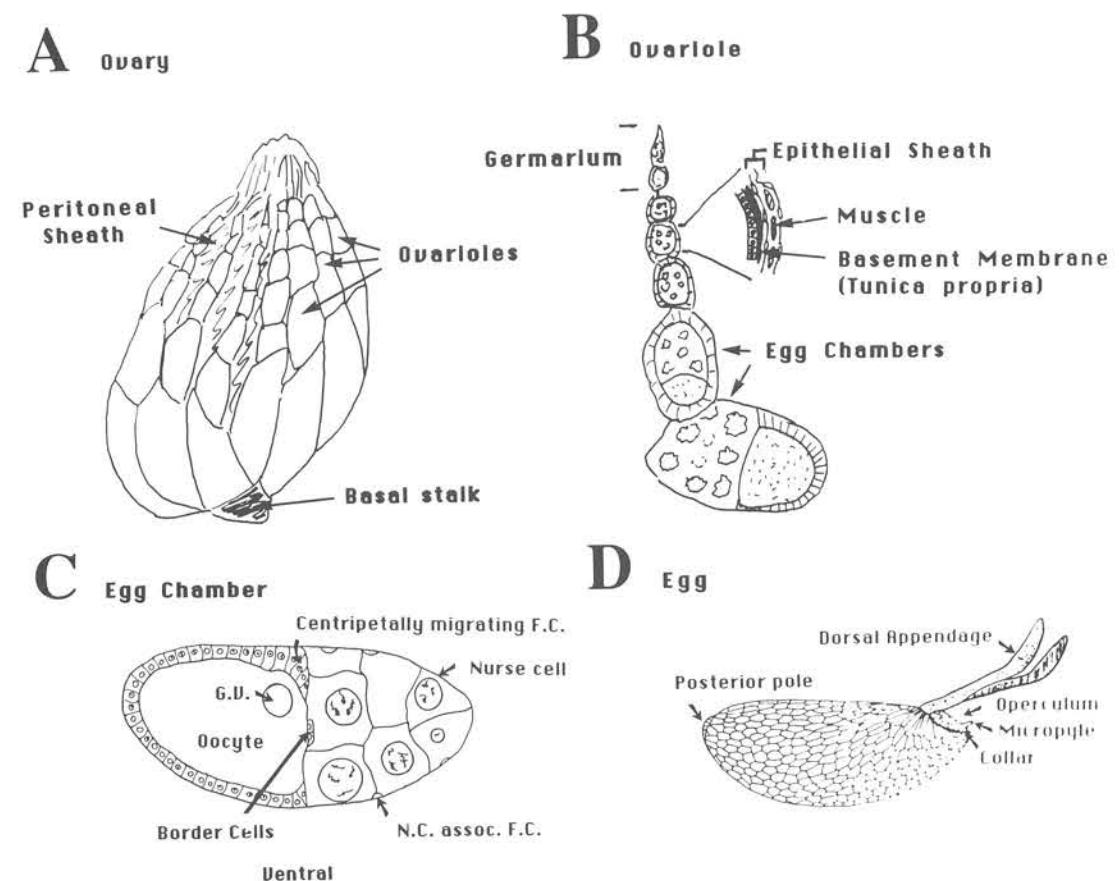


Figure 1 Morphology and terminology. Drawings depict an entire ovary (A) and ovariole (B), a stage-10 egg chamber in cross section (C), and a mature egg (D). Anatomical terms used in the text are indicated. (B) Multi-layered wall lining of each ovariole in cross section. A layer of follicle cells, the overlying basement membrane, and the two layers of the epithelial sheath sandwiching muscle fibers are shown. A was redrawn from Mahowald and Kambysellis (1980); C and D were modified from Parks and Spradling (1987).

II. BACKGROUND

This section briefly reviews the structure and physiology of egg production to assist readers without prior experience with the *Drosophila* ovary.

A. Anatomy and Terminology

A typical ovary (Fig. 1A) contains about 16 ovarioles, each representing an independent egg assembly line. Every ovariole (Fig. 1B) is tipped with germ-line and somatic stem cells whose progeny are subsequently organized into egg chambers (follicles) within a distinct anterior region called the germarium. Egg chambers leave the germarium and continue developing as they move posteriorly within the ovariole. Ovarioles usually contain six to seven sequentially more mature follicles, each separated by short chains of interfollicular stalk cells. Each egg chamber string lies within an ovariolar wall (and associated tissues) that helps to maintain the linear order of developing chambers and

moves them posteriorward with time. Working outward, each egg chamber is immediately surrounded by a basement membrane (or tunica propria). The integrity of the ovariole derives from a thin covering of muscles sandwiched between two epithelial layers, which together are called the epithelial sheath. Finally, the ovarioles are held together by a sparser network of connective tissue—the peritoneal sheath—that includes abundant tracheoles to pipe in the oxygen needed for ovarian metabolism. Ovarian muscles contract rhythmically, an activity that probably helps the hemolymph circulate and moves egg chambers posteriorly whenever any space is created by the entry of an egg chamber into the oviduct. In addition, the ovary contains nervous tissue (poorly characterized) and, near the junction with the oviduct, basal stalk cells

abundant in lysosomes that probably aid in recycling mature follicle cells and degenerating egg chambers.

Individual egg chambers that have left the germarium consist of three cell types (Fig. 1C). The oocyte with its prominent nucleus (or germinal vesicle, GV) is connected to 15 nurse cells; both are surrounded by a monolayer of approximately 1000 follicle cells. In egg chambers that have developed to the point illustrated in Figure 1C, distinct subpopulations of follicle cells can be recognized. Six to ten border cells migrate between the nurse cells to reach the anterior end of the oocyte, where they are soon joined by a group of centripetally migrating follicle cells. About 50 cells remain associated with the nurse cells. The completed egg is covered with an eggshell (chorion) containing several prominent features (Fig. 1D). Two dorsal appendages (filaments) that facilitate embryonic respiration protrude from the anterior end. A much smaller region of specialized chorion is found at the posterior pole that in some insects serves a similar purpose. Just below the dorsal filaments, a horseshoe-shaped ridge known as the collar defines the borders of the larval exit door or operculum. A single cone-shaped micropyle provides a pathway for sperm entry prior to fertilization.

Stage	Length	Identification	Nomarski	DAPI
2	8 hr	average size: 25x25 μ ; karyosome has not yet formed (arrow); oocyte and nurse cell nuclei similar in size		
3	8 hr	average size: 35x35 μ ; karyosome (k) and endobody are visible in germinal vesicle		
4	6 hr	average size: 40x50 μ ; nurse cell nuclei contain similar amounts of DNA and appear polytene (arrow)		
5	5 hr	average size: 55x75 μ ; nurse cell nuclei no longer polytene (arrow); posterior nurse cells have more DNA than anterior ones		
6	3 hr	average size: 60x85 μ ; follicle cell mitoses cease; nurse cell ploidy equal		
7	6 hr	average size: 70x115 μ , more elongated in shape than previous oval-shaped stages; nurse cells have higher ploidy at posterior; no yolk visible in oocyte		
8	6 hr	yolk visible in oocyte (arrow); follicle cell layer still uniform		

Figure 2 (Continued on facing page.) Stages of oogenesis: a field guide (see King 1970; Mahowald and Kambyrellis (1980).

Stage	Length	Identification	Nomarski	DAPI
8	6 hr	yolk visible in oocyte; follicle cell layer uniform		
9	6 hr	follicle cells in process of migration over oocyte, resulting in an anterior-posterior gradient of cell thickness (arrows); oocyte is about 1/3 of egg chamber		
10A	6 hr	follicle cells form columnar epithelium over oocyte; centripetal migration not yet visible; oocyte is 1/2 of egg chamber		
10B	4 hr	centripetal migration in progress (arrows); dorsal follicle cells thicker than ventral (triangle); vitelline membrane extends into opercular region		
11	0.5 hr	oocyte larger than nurse chamber due to onset of nurse cell dumping, but dumping still incomplete		
12	2 hr	dumping complete; 15 nurse cell nuclei remain at anterior; dorsal filaments not visible in nurse chamber		
13	1 hr	some nurse cell nuclei still remain; dorsal filaments visible at anterior end		
14	>2 hr	no nurse cell nuclei remain; dorsal filaments complete their elongation		

¹ based on King, 1970; Mahowald and Kambyrellis, 1980, and Lin and Spradling, unpublished. Duration of stages estimated from the development of stage 1 egg chambers transplanted into host females. Photographs, courtesy of Dr. H. Lin.

Egg chamber development has been divided on morphological grounds into 14 stages (Fig. 2), whose approximate duration was inferred from their relative abundance in the ovaries (King 1970; Mahowald and Kambyrellis 1980). This system works well beginning with stage-8 egg chambers, when events associated with yolk deposition, follicle cell migration, nurse cell breakdown, and eggshell synthesis allow successively older chambers to be readily recognized. Most later stages could be subdivided further on the basis of these unique mileposts. In contrast, living previtellogenic egg chambers are difficult to distinguish. Figure 2 provides a key to recognizing egg chamber stages. Identification is facilitated by staining the chambers with DAPI to reveal DNA. Recently, the duration of oogenetic stages has been reexamined (H. Lin and A. Spradling, in prep.). The development to maturity of stage-2 egg chambers was monitored following transplantation into a host female lacking functional ovaries. Under these conditions, the total time between stage 1 and 14 and the early stages proceeded faster than previous indirect measurements, but vitellogenesis stages appeared to last longer. These differences probably reflect the faster development of a single egg chamber in the absence of nutrient limitations that occur in a normal ovary.

B. Ovarian Physiology

Egg chambers, unlike embryos, do not develop as a closed system in relative isolation from environmental variables. Consequently, it is essential for researchers wishing to examine any aspect of oogenesis to appreciate the basic processes regulating ovarian function. The ovary changes with maternal age and is extremely sensitive to the available nutritional and environmental resources. Consequently, ovaries can be so small that they are difficult to locate, or they can constitute more than half the body weight, swelling females to nearly twice the size of their male siblings. If newly eclosed females are continuously provided with an excess of food (e.g., moist live yeast), the ovaries rapidly develop from their relatively immature state at eclosion to a "steady-state" condition that can last more than 1 week, followed by an age-dependent decline in egg production. The same basic process of egg development appears to occur under less favorable conditions, although the number of egg chambers that mature may be greatly reduced. Statements of developmental time assume that egg chambers are developing under ideal conditions. Little is known about the arrest points and feedback loops that coordinate oogenesis under the nonoptimal conditions that flies usually encounter.

The onset of yolk production (vitellogenesis) at the beginning of stage 8 constitutes one control point that is relatively well understood. Since the oldest chambers at eclosion are in stage 7, more than 24 hours are required to produce the first mature egg. However, vitellogenesis in young females initially utilizes nutrients stored in the larval fat cells, since feeding does not commence for 12–24 hours. Both ecdysone and juvenile hormone are involved in turning on vitellogenesis in young females (see Postlethwait and Kunert 1986; Bownes 1989). Moreover, if an adequate supply of protein is not available to the female, yolk is produced at low levels and egg chambers progress into stage 8 at a retarded rate regardless of age. Oogenesis under such limiting conditions may resemble the defects seen in mutants unable to produce large amounts of protein due to defects in rRNA (*bobbed* mutants, Mohan 1971;

Mermod and Crippa 1978), 5S RNA (*min* mutants, Procnier and Dunn 1978), ribosomal proteins (certain *Minutes*, see Walker 1985), or old flies (see Lints and Soliman 1988). Slower egg production allows some degree of fertility to be salvaged.

Oviposition of mature eggs, a process controlled by an "egg-laying hormone" peptide in other invertebrates, provides a second major control point. Since immature eggs are never laid, feedback mechanisms must exist that prevent stage-14 egg chambers from precociously entering an oviduct prior to completion of the ovary phase of oogenesis. *Drosophila* females are loath to deposit even mature eggs unless relative humidity, presence of nutrients, and seemingly idiosyncratic factors signal the presence of an environment hospitable for larval development. Even eggs held for 1 week or more after maturity usually develop normally once the female is provided with the missing conditions. When mature eggs are not laid, the ovarioles "back up" as each ovariole acquires two or even three stage-14 egg chambers. Progression of egg chambers into vitellogenesis becomes blocked, and egg chamber stages between stage 7 and 14 disappear. If not laid, mature egg chambers in backed-up ovarioles are eventually resorbed.

Another feedback control on oviposition signals the presence of seminal fluid in the seminal receptacle or spermathecae of the female. Unfertilized females lay few eggs and their ovaries back up. Eventually, some eggs are laid; however, their quality is poor and they quickly show signs of gross degeneration (see Bouletreau-Merle et al. 1989). Since mating with a sterile male can overcome this block, a specific signal transmitted in the seminal fluid (rather than sperm per se) is postulated to signal that mating has occurred. Male-specific peptides produced in the male accessory gland are candidates for such a signal (Chen et al. 1988). Less is known about how the depletion of sperm is monitored once it has been stored, but females become receptive to males when their supply of sperm runs low.

Because of these controls, it is essential to examine mutant ovaries under optimal conditions: in well-fed 2–4-day-old females. Some mutant strains, whose ovaries contain defective egg chambers, do produce and lay a small number of morphologically normal eggs. A biased sample of mutant defects is likely to be observed if only laid eggs or backed-up ovaries are examined. Egg chambers in old flies may more frequently break down and be resorbed or spontaneously fuse with adjacent egg chambers.

III. EXPERIMENTAL STRATEGIES

Progress in understanding oogenesis is tied to the methods available to identify genes whose function is important for egg production. The section below therefore considers these methods and their limitations. Our current knowledge of the genes likely to have specific functions during oogenesis is summarized in Tables 2 through 4. Genes that are active during oogenesis, but whose products do not function until after fertilization, have been excluded. Only well-characterized genes (usually with more than one allele) are included, unless justified by additional studies. Additional genes are listed based solely on molecular or biochemical studies if they are known to have a unique function during oogenesis.

Table 1 Female Sterile Screens

Screen	Number	Viable	Sterile	Comments
Gans et al. (1975)	2,700 ^a	1,064	95	EMS
Mohler (1977)	16,200 ^a	5,524	324	EMS
Komitopoulou et al. (1983)	3,000 ^a	1,071	87	EMS
Schüpbach and Wieschaus (1989, 1991)	18,782	7,351	529	EMS
Erderly and Szabad (1989)	50,128	50,128	51	EMS (dominants only)
Szabad et al. (1989)	18,723	18,723	24	EMS (dominants only)
Spradling et al. ^b	13,400	11,901	250	single P insert lines

^aEstimated from percent lethality.

^bCombined from five screens: (1) Cooley et al. 1988; (2) Berg and Spradling 1991; (3) L. Yue, C. Berg, and A. Spradling, unpubl.; (4) D. Stern and A. Spradling, uppubl.; (5) C. Berg, L. Cooley, R. Glaser, B. Harkins, M. Heck, G. Karpen, R. Kelley, M. Kuhn, L. Lee, D. McKearin, D. Montell, T. Oyebode, J. Riesgo, D. Sommerville, D. Stern, D. Thompson, J. Tower, E. Verheyen, S. Wasserman, L. Yue, and A. Spradling (see Karpen and Spradling 1992).

A. Genetic Methods

The potential to dissect oogenesis genetically provides the major reason to study this process in *Drosophila*. There is no shortage of raw materials for such an analysis. Large collections of sterile or lethal mutations have been constructed that undoubtedly disrupt many genes necessary for oogenesis. However, the role of genes in egg development has frequently been difficult to deduce from mutant phenotypes. Effects on oogenesis are often masked by lethality or clouded by the production of variable or nonspecific defects. More experience is needed to separate genes with functions unique to oogenesis (referred to as "important" genes) from those required only indirectly, for example, to support intermediary metabolism. Consequently, much effort is currently directed at identifying key genes involved in the major developmental processes of egg development.

1. Female Sterile Mutations

Female sterile mutations are the simplest and most direct source of potentially important oogenetic genes. In 1929, Bridges discovered the first gene whose only known phenotype was female infertility. In one of his less perspicacious moments, Bridges named it simply *female sterile* (*fes*), now *fs(2)B*. A long period during which genes required for ovarian function were only identified sporadically (usually by virtue of an associated visible phenotype) ended with the advent of large-scale screens for female sterile mutations (Table 1). Surprisingly, most of the more than 1300 female sterile mutations that have been analyzed fall into a small number of phenotypic categories (see Fig. 3). (For convenience, specific genes are listed by chromosomal position [Tables 2-4]. Thus, in the text, genes are followed by their approximate cytogenetic location to facilitate cross-referencing.)

The earliest defects are caused by **rudimentary** mutations (*stonewall* [70C], progeny of *osk*³⁰¹ [85B] mothers). The ovaries in such flies are extremely small (Fig. 3A); germaria are difficult to identify and characterize, and in many cases, germ-line cells are completely lacking. **Tumorous** mutants also develop

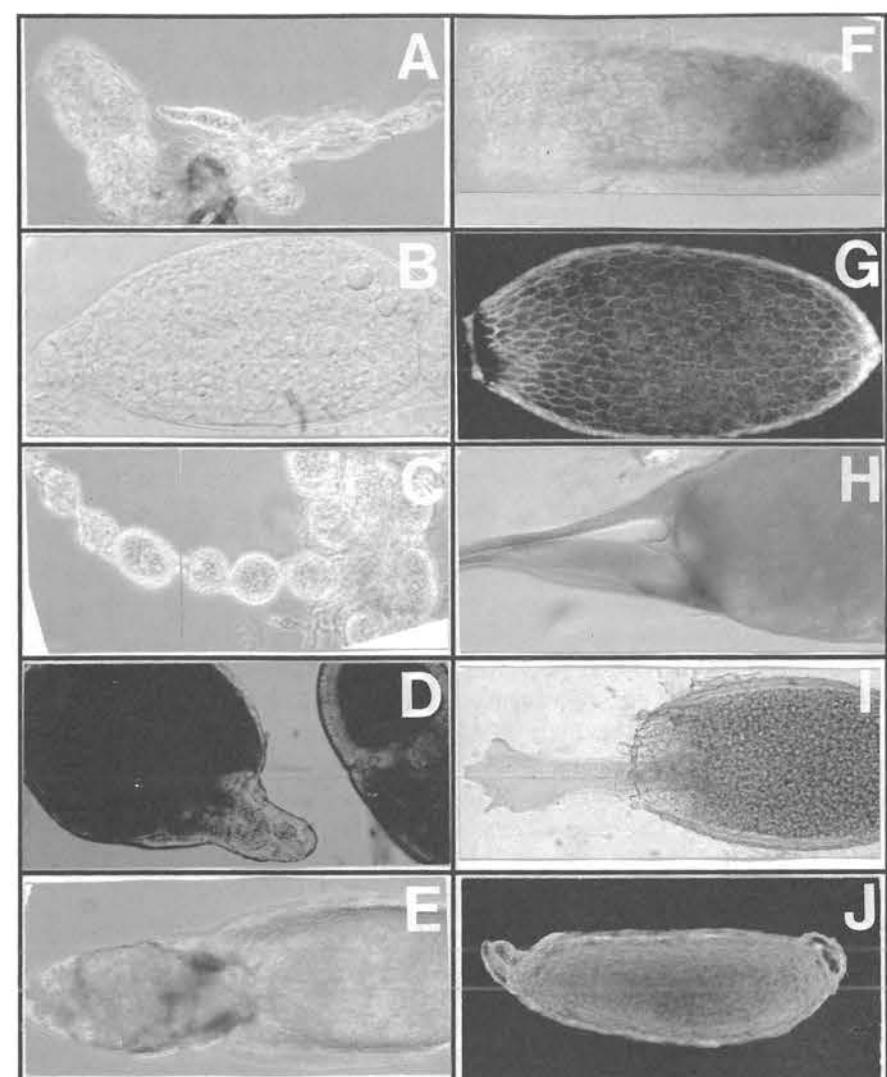


Figure 3 Phenotypes of female sterile mutations. Phase-contrast or Nomarski micrographs of living unfixed egg chambers are illustrated, except in G, which was fixed and cleared to illustrate the chorion pattern. (A) An entire ovary; (B-C) a single ovariole; (D-J) egg chambers representing the terminal stage reached by the mutant in question. The name of the mutant class and the genotype of the homozygous females producing the egg chambers are given for each panel. (A) Rudimentary: *stn*³⁴¹⁷; (B) tumorous: *orb*¹⁰⁷; (C) degenerating: 2335 (H. Lin and A. Spradling, pers. comm.); (D) small egg: 6448 (A. Spradling et al., pers. comm.); (E) dumpless: *chickadee*⁴⁶¹²; (F) cup-shaped: *cup*¹³⁵⁵; (G) ventralized: *gurken*^{WG42} (Montell et al. 1991); (H) dorsalized: *squid*⁰⁰⁴⁸; (I) fused filament: rare *nanos*⁵⁶⁷⁸/+ chamber; (J) thin chorion (or collapsed): *chiffon*^{WF24} (Schüpbach and Wieschaus 1991; photo courtesy of J. Tower). Magnifications: (A,C,E,F,I) 150x. (B) 750x; (D,G,H) 225x; (J) 112x.

small, poorly differentiated ovaries (*otu* [7F], *fs(2)B* [22]). The strongest tumorous mutations prevent egg chamber formation. Instead, the ovarioles contain an excess of small, disorganized cells in the tip region (Fig. 3B). In other tumorous mutants, a variable number of abnormal egg chambers assemble that contain anywhere from tens to many hundreds of small cells. (The design-

nation tumorous is not particularly appropriate for these mutants, since masses of cells do not grow to large size and will not proliferate when transplanted, for example, into the abdominal cavity.) Some "pseudonurse" cells within these tumorous chambers grow extensively and resemble normal nurse cells.

Degenerating mutations do not prevent egg chamber formation. However, the follicles produced by homozygotes develop to only a limited extent before undergoing degeneration, often at a characteristic stage of development and following a prolonged period of developmental arrest (Fig. 3C). They probably represent a diverse group of genes. Mutants in which either the follicle cells (*suvar(2)1* [31A]) or the nurse cells (*su(Hw)* [88B]) degenerate have been described, but nurse cell degeneration appears to be more common. The egg chambers in degenerating mutants frequently show defects long before they completely disintegrate. In **egalitarian** mutants (*egl* [59F], *Bic-D^r* [36C]), 16 nurse cells are present but no oocyte. Other degenerating mutants produce chambers containing a variable number of nurse cells but rarely or never an oocyte.

Flies bearing many other female sterile mutations have egg chambers that progress through oogenesis, but the resulting eggs are highly abnormal. Characteristic pattern defects are often evident in the structure of the eggshells produced by such follicles. **Small egg** mutants (*seg* [19F], *kugeli* [84]) produce very small, round eggs no larger than stage-10 oocytes (Fig. 3D). **Cup or open chorion** mutations (*cup* [27A], *chalice* [32]) cause eggs to develop that completely lack anterior chorions (Fig. 3F). The follicle cells in such chambers secrete eggshell over both the abnormally small oocyte and several adjacent nurse cells. The nurse cells of flies with **dumpless** mutations (*sn* [7D], *chk* [26A]) are unable to transfer their contents to the oocyte or do so incompletely (Fig. 3E). The untransferred nurse cell cytoplasm remains at the anterior end of the oocyte where it appears to interfere with the ability of the follicle cells to produce dorsal filaments.

Several classes of mutants disrupt the pattern of the eggshell or the internal structure of the oocyte in a manner suggesting that defects are associated with a particular developmental coordinate system. **Dorsalized** mutants (*K10* [2E], *spire* [37E]) produce egg chambers lacking most of the signs of dorsal-ventral polarity that normally appear beginning in stage 9 (Fig. 3H). Eggs are produced that contain a cylinder of chorion all around the anterior end, instead of two dorsal appendages; expression of this phenotype is variable in some mutants. Eggs from **ventralized** mutants (*gurken* [29C], *torpedo* [57F]) lack dorsal appendages and have a characteristic syndrome of eggshell changes (Fig. 3G). Other mutants cause variable disruptions of egg structures along the dorsal-ventral axis; a direct role for these genes in pattern formation remains uncertain. **Posterior** group genes were originally defined by their effects on embryonic segmentation and pole cell formation. Several of these genes (*nanos* [91F], *vasa* [35B]) may have other functions in oogenesis in addition to forming the oocyte polar plasm.

A variety of little-studied mutants appear to primarily affect the eggshell. **Fused filament** mutants (*rhino* [54C], *unicorn* [63D]) produce only a single dorsal appendage along the dorsal midline (Fig. 3I). Other filament defects result in short or deformed filaments. Often, both fused filament and short filament phenotypes are variable in penetrance. Similar defects are seen in a fraction of eggs produced by mutants in other classes, for example, by posterior mutants.

Eggs produced by **thin chorion** mutations (*chiffon* [35F], *gauze* [2-74]) have much less chorion than normal (Fig. 3J). **Collapsed egg** mutants (*Nasrat* [2A], *QP42* [26A]) produce normal-looking stage-14 eggs that collapse when held in the ovary or following oviposition. Frequently, there is no evidence that the laid eggs were ever fertilized.

Several additional classes of mutations are not considered in detail, because they probably do not define genes required for egg development to stage 14. **Held-egg** mutants cause females to retain apparently normal, mature stage-14 egg chambers. Egg accumulation in certain *AbdB* alleles is so great that females swell dramatically (L. Schneider and A. Spradling, pers. comm.). Little is known about this class, which may include genes involved in controlling egg maturation and other functions occurring within the oviducts and uterus. A large fraction of **maternal effect lethal** genes encode products that only function after fertilization. The properties of these genes are reviewed elsewhere.

Female sterile mutations were obtained at about the same rate (relative to lethal mutations) using *P* elements or ethylmethanesulfonate (EMS) (Table 1). However, the spectrum of sterile mutations was different with the two mutagens. About 12% of EMS-induced mutations affected early oogenesis, 63% produced morphological defects at later stages, and 25% were maternal effect lethals (Schüpbach and Wieschaus 1991). In contrast, nearly 50% of single *P*-element sterile mutants produced normal stage-14 egg chambers, whereas only 36% resulted in mid to late morphological defects. This difference probably reflects the fact that many *P* insertions create hypomorphic alleles and possibly also the much greater site selectivity of *P*-element mutagenesis.

Complementation testing suggested that about half the mutants isolated in female sterile screens are rare hypomorphic alleles of vital genes (Perrimon et al. 1986; Schüpbach and Wieschaus 1991). Thus, only a subset of the genes defined by sterile mutations, sometimes called "true" steriles, correspond to genes that repeatedly mutate to female sterility. Many true steriles probably function exclusively in the ovary; however, it is already known that others also have vital functions despite their ready mutation to female sterility. Extrapolating from EMS screens of the X and second chromosome, where average allele frequencies as high as 3.0 have been reported, approximately 200 true steriles (excluding maternal effect lethals) are expected throughout the entire genome (Perrimon et al. 1986; Schüpbach and Wieschaus 1991). More than 100 such loci are listed in Tables 2 through 4.

2. Lethal Mutations

Many genes perform essential functions prior to adult development. Null mutations in such genes provide no insight into their role in oogenesis. The easiest way to recognize that vital genes function during oogenesis has been through the analysis of hypomorphic or conditional alleles. Some of the rare sterile alleles of lethals undoubtedly do reveal an interesting and important role for the gene in egg development. For example, the *torpedo* gene encoding a *Drosophila* epidermal growth factor (EGF) receptor tyrosine kinase that transduces signals required for egg and embryonic dorsal-ventral differentiation was identified by a single sterile allele. Stronger mutations are embryonic lethals originally named "faint-little-ball" (Clifford and Schüpbach 1989; Price et al. 1989; Schejter and Shilo 1989). Despite other examples of important lethals

Table 2 X Chromosome Genes Involved in Oogenesis

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>Nasrat</i> (N)	2A4	10	G	col; MAT (terminal) lethal; dump, small; plakoglobin-like protein early ecdysone response	Perrimon and Gans (1983); Perrimon et al. (1986); Degelmann et al. (1990)
<i>armadillo</i> (<i>arm</i>)	2B15				Wieschaus and Noell (1986); Riggelman et al. (1990)
<i>Broad-complex</i> (<i>fs(1)del2</i>)	2B	1	S		Galanopoulos et al. (1989); Shea et al. (1990)
<i>ultraspiracle</i> (<i>usp</i>)	2C4-8	4		lethal; = CF1 steroid receptor protein	Shea et al. (1990); Oro et al. (1990)
K10	2E3		G	dors; GV nuclear protein	Wieschaus et al. (1978); Wieschaus (1979); Haenlin et al. (1987); Proust et al. (1988); Cheung et al. (1992)
<i>nel</i>	0-0.5-5	8	2	cor	Galanopoulos et al. (1989)
<i>Ya</i>	3B4-6	3	G	MAT; defective pronuclear synamy	Perrimon et al. (1986); Lin and Wolfner (1989, 1991)
<i>Yb</i>	3B4-6	3		rud, tum	Lin and Wolfner (1991); H. Lin (pers. comm.)
<i>Notch</i> (N)	3C7	50	S	lethal, chambers fail to bud	Rouhola et al. (1991); T. Xu and S. Artavanis-Tsakonas (pers. comm.)
<i>diminutive</i> (<i>dm</i>)	3C9-D2	2		degen	King (1970)
<i>dunce</i> (<i>dnc</i>)	3D4	6	G	col	Bellen and Kiger (1988); Bellen et al. (1987)
<i>brainiac</i> (<i>brn</i>)	3F7-4A6	2	G	prefollicular migration dors/vent	Goode et al. (1992)
<i>small egg</i> (<i>se</i>)	4B1-C2			small	Mohler (1977)
<i>Mei-9</i>	4C1-5	10		mei	Carpenter (1982); Curtis and Bender (1991)
<i>ovo</i>	4C15-F1	20	G	rud, tum; Zn finger protein	Busson et al. (1983); Mevel-Ninio et al. (1989, 1991); Oliver et al. (1987, 1990)
K1214	5D5-E1	1	S	thin; def chorion amplification	Perrimon and Gans (1983); Orr et al. (1984)
<i>sans fille</i> (<i>snf</i>) = <i>liz</i>	4F	1	G	tum; interacts with <i>Sxl</i>	Gollin and King (1981); Oliver et al. (1988); Steinmann-Zwicky (1988); Salz (1992)
<i>fs(1)pole</i> <i>hole</i> (<i>phe</i>)	5C5-D6	7	G	col; MAT (terminal)	Perrimon and Gans (1983); Degelman et al. (1986, 1990)
M13	5C7-D6	8			Mohler (1977); Lindsley and Zimm (1985, 1990)
<i>swallow</i> (<i>swa</i>)	5E6-E7	6	G(?)	col ooc; required for bicoid localization	Frohnhofer and Nüsslein-Volhard (1987); Stephenson et al. (1988); Chao et al. (1992)
<i>Sex-lethal</i> (<i>Sxl</i>)	6F5	20	G,S	lethal, tum; RNA-binding protein?	Cline (1983, 1984); Schüpbach (1985); Steinmann-Zwicky et al. (1989)
<i>dec1</i>	7C1	20	S	chor	Wieschaus et al. (1981); Lineruth and Lambertsson (1986b); Waring et al. (1990)
<i>singed</i> (<i>sn</i>)	7D1	50	G	dump	King (1970); Patterson and O'Hare (1991)
<i>Cp38</i> , four others	7F1	0	S?	chorion structural proteins	Spradling (1981); Spradling et al. (1987); Kerramaris et al. (1991)
<i>Cp36</i> (<i>cor-36</i>)	7F1	3	S	thin; chorion structural protein	Digan et al. (1979); Underwood and Mahowald (1980); Tolias and Kafatos (1990)
<i>ovarian tumor</i> (<i>otu</i>)	7F2	20	G	tum	King and Storto (1988); Steinhauer et al. (1989); Steinhauer and Kalfayan (1992)
M112	7E10-8A5	2		degen (S8/9)	Mohler (1977); Lindsley and Zimm (1985, 1990)
M49	9A	3		col	Mohler (1977); Lindsley and Zimm (1985, 1990)
<i>YPI</i> (<i>fs(1)1163</i>)	9A-B	1	S	ooc, col, da; yolk protein	Giorgi and Postlethwait (1985); Saunders and Bownes (1986); Logan et al. (1989)
<i>YP2</i> (<i>fs(2)A59</i>)	9A-B	3	S?	ooc, col, da; yolk protein	Perrimon and Gans (1983); Logan et al. (1989)
M116	9E3-10A1	2		degen (S8)	Mohler (1977); Lindsley and Zimm (1985, 1990)
<i>haplo female</i> <i>sterile</i> (<i>hs</i>)	10A8-A11	1		MAT	Lindsley and Zimm (1985, 1990)

(continued on following page)

Table 2 (Continued)

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>no distributive disjunction (nod)</i>	10C2	8	G?	mei; kinesin-like protein	Hawley (1988); Zhang et al. (1990)
<i>tiny (ty)</i>	12A6-D3	1	S	degen (S8/9)	King (1970); DiMario and Hennen (1982); Perrimon and Gans (1983)
120	1-47	6	S	MAT	Perrimon and Gans (1983)
<i>mus101</i>	12B2-8	6	G,S	lethal, thin	Gatti et al. (1983)
<i>YP3 (A1526)</i>	12B-C	1	S?	fertile, eliminates YP3 in egg; yolk protein	Garabedian et al. (1987)
<i>yolkless</i>	12E	30	G	ooc; yolk uptake reduced	Waring et al. (1983); DiMario and Mahowald (1987)
<i>mei41</i>	14C	15		mei	Hawley (1988); Mason et al. (1989)
<i>Ars</i>	15D1	1		mei	Zitron and Hawley (1989)
<i>fused (fu)</i>	17C4-C6	30	G	tum, lethal; putative Ser-Thr kinase	King (1970); Perrimon et al. (1986); Busson et al. (1988); Preat et al. (1990)
<i>A273</i>	18E1-20A	3	S	da	Komitopoulou et al. (1983); Perrimon et al. (1986)
<i>short egg (seg)</i>	19F	1	S	small	Wieschaus (1978); Weischaus et al. (1981); Perrimon et al. (1989b)
<i>suppressor of forked (su(f))</i>	20EF	20		MAT	Wilson (1980); Gutzeit and Heinrich (1981); Lineruth and Lambertsson (1986a)

^{a,b}See Lindsley and Zimm (1985, 1990).^cMinimum number of alleles.^dAutonomous to S (soma) or G (germ line). ? indicates based on expression data only.^ePhenotypic abbreviations: col (collapsed); cor (defective chorion; flaccid); cup (cup-shaped); da (defective dorsal appendages); degen (degenerating); dors (dorsalized); dump (dumpless); GV (germlinal vesicle); MAT (maternal effect); ms (male sterile); mei (metotic defects); ooc (defective oocyte); rud (rudimentary); S (stage); small (small egg); sl (semi-lethal); thin (thin chorion); tum (tumorous); var (variable); vent (ventralized).

Table 3 Second Chromosome Genes Involved in Oogenesis

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>okra (okr)</i>	2-1	2		var dors/vent	Schüpbach and Wieschaus (1991)
<i>gourd (gou)</i>	2-5	2		var dors/vent	Schüpbach and Wieschaus (1991)
<i>fs(2)B</i>	2-5	1		tum	King (1970)
<i>I(2)giant larvae (lg)</i>	21A1-C1	20		tum, lethal	King (1970); Mechler et al. (1985)
<i>Star (S)</i>	21D2-22A1	G			Mayer and Nüsslein-Volhard (1988)
<i>cappuccino (capu)</i>	24D	2	G	var dors, ooc	Manseau and Schüpbach (1989b)
<i>Sv23, two minor</i>	26A	0	S?	vitelline membrane protein cluster	Mindrimos et al. (1985); Podopri et al. (1988)
<i>vn proteins</i>				col; 17.5-kD vitelline membrane protein in cluster	Savant and Waring (1989)
<i>Sv17.5 (fs(2)QP42)</i>	26A	1	S?	membrane protein in cluster	Schüpbach and Wieschaus (1991); Cooley et al. (1992)
<i>chickadee (chic)</i>	26A	10	G?	dump; profilin	Schüpbach and Wieschaus (1991); A. Spradling (unpubl.)
<i>cup (cup)</i>	27A/B	41		degen, cup	Schüpbach and Wieschaus (1991)
<i>muslin (mln)</i>	2-28	2		col	Schüpbach and Wieschaus (1991)
<i>gurken (grk)</i>	29C	7	G	vent	Schüpbach (1987)
<i>string of pearls (sop)</i>	30D/E	1		degen	Crampton et al. (1992)
<i>Su(var)2-1</i>	31A2-C	5		degen	Dorn et al. (1986); Lindsley and Zimm (1990)
<i>beads-on-a-string (boas)</i>	31B	1		degen	Cooley et al. (1988); L. Yue and A. Spradling (pers. comm)
<i>flipper (flp)</i>	31B	2		tum, visible	Lindsley and Zimm (1985); Berg and Spradling (1991); deValoir et al. (1991)
<i>chalice (chal)</i>	2-37	3		cup, da	Schüpbach and Wieschaus (1991)
<i>aubergine (aub)</i>	2-39	5		var dors/vent	Schüpbach and Wieschaus (1991)
<i>piwi (piwi)</i>	32C	3		rud, degen; also ms	H. Lin, P. Zhang, and A. Spradling (pers. comm.)
<i>abnormal oocyte (abo)</i>	32E	1		ooc	Sandler (1977); Sullivan and Pimpinelli (1986); Lavorgna et al. (1989); Tomkiel et al. (1992)
<i>Vm32E</i>	32E	0	S?	13-kD vitelline membrane protein	Gigliotti et al. (1989)

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Table 3 (Continued)

Gene ^a	Site ^b	Alleles ^c	Aut ^d	Information ^e	References
<i>arrest (aret)</i>	33B3-F1,2	8		rud, degen; also ms var dors/vent	Schüpbach and Wieschaus (1991)
<i>zucchini (zuc)</i>	33B3,7-F2	3		vitelline membrane protein	Schüpbach and Wieschaus (1991)
<i>Vm34C</i>	34C	S?		ooc, MAT (posterior);	Mindrinos et al. (1985); Scheer et al. (1988)
<i>vasa (vas)</i>	35B	10	G?	DEAD protein family	Schupbach and Wieschaus (1986a); Lasko and Ashburner (1988, 1990); Hay et al. (1988a,b, 1990)
<i>twine</i>	35F	1		mei	Courtot et al. (1992)
<i>chiffon (chf)</i>	35FI-36A1	8		thin; defective gene amplification	Ashburner et al. (1990); Schüpbach and Wieschaus (1991); J. Tower and A. Spradling (pers. comm.)
<i>cornichon</i>	35FI-36A1			vent	Ashburner et al. (1990)
<i>midway (mdy)</i>	2-53	2		degen (S8/9)	Schüpbach and Wieschaus (1991)
<i>squash (squ)</i>	2-53	3		var dors/vent	Schüpbach and Wieschaus (1991)
<i>Bicaudal-C (Bic-C)</i>	35EI-E2	5		degen, cup	Mohler and Wieschaus (1986); Schüpbach and Wieschaus (1991)
<i>quail (qua)</i>	36C8-11	6		dump	Steward and Nüsslein-Volhard (1986); Schüpbach and Wieschaus (1991)
<i>kelch (kel)</i>	36E1-E3	8		cup, dump	Steward and Nüsslein-Volhard (1986); Yue and Cooley (1990); Schüpbach and Wieschaus (1991)
<i>Bicaudal-D (Bic-D)</i>	36C8-C11	4	G?	degen; blocks oocyte determination	Steward and Nüsslein-Volhard (1986); Suter et al. (1989); Wharton and Struhl (1989); Suter and Stewart (1991)
<i>fasciclin III</i>	36EI	2	S?	fertile; cell adhesion protein	Patel et al. (1987); D. Montell (pers. comm.)
<i>spire (spir)</i>	37E3-F1	4	G	var dors, ooc	Manseau and Schüpbach (1989b)
<i>spitzi (spi)</i>	37E2-38E	2	G		Mayer and Nüsslein-Volhard (1988)
<i>deadlock (del)</i>	38A6-E9	4		rud, da	Schüpbach and Wieschaus (1986)
<i>valois (val)</i>	38B6-C2	4		ooc, MAT (posterior)	King (1970); Wilson (1981); Cohen et al. (1992)
<i>apterous (ap)</i>	41B-C	30		degen (S7/8); visible	H. Lin, V. Siegel, C. Berg, and A. Spradling (pers. comm.)
<i>zeppelin (zep)</i>	47A9-10	4	S?	degen, var dors/vent, ooc	Schüpbach and Wieschaus (1991)
<i>crepe (cre)</i>	2-59	6		col	Schüpbach and Wieschaus (1991)
<i>cut off (cuff)</i>	61	3		rud, degen; also ms	Schüpbach and Wieschaus (1991)
<i>stand still (stii)</i>	48D3-49B3	2		rud, degen, nurse cell number	Schüpbach and Wieschaus (1991)
<i>bicaudal (bic)</i>	49D3-E6	1		MAT, ant/post	Lindsley and Zimm (1990)
<i>PA77</i>	51F	2		thin, lethal; defective chorion amplification	Underwood et al. (1990); Schüpbach and Wieschaus (1991)
<i>gauze (gau)</i>	2-74	7		thin	Schüpbach and Wieschaus (1991)
<i>stopped</i>	2-76	2		rud, sl	Schüpbach and Wieschaus (1991)
<i>closedown (clo)</i>	2-77	3		degen	Schüpbach and Wieschaus (1991)
<i>transformer-2 (tra-2)</i>	51B4-B6	10		sex determination	Belote et al. (1985); Kraus et al. (1988)
<i>narrow</i>	2-83	2		tum, sl, visible	King (1970)
<i>windbeutel (wind)</i>	2-86	1	S	MAT, dors/vent	Schüpbach and Wieschaus (1989)
<i>rhino</i>	54C	2		da	Berg and Spradling (1991); C. Berg (pers. comm.)
<i>adipose (adp)</i>	55A-C2	2		ooc, visible; yolk reduced	King (1970)
<i>halted (hal)</i>	55A-C	3		rud, MAT	Schüpbach and Wieschaus (1989); Berg and Spradling (1991); St. Johnston et al. (1991)
<i>staufen (stau)</i>	55A1,2	5	G	ooc, MAT (posterior)	Yue and Spradling (1992)
<i>hu-li tai shao (hts)</i>	56C/D	5	G?	degen, visible; nurse cell number; adducin	Frohnhofer and Nüsslein-Volhard (1987); Hazelrig et al. (1990)
<i>exuperantia (exu)</i>	57A2-B1	7	G	ooc, MAT	Boswell et al. (1992)
<i>mago nashi (mago)</i>	57C	3	G?	ooc, MAT (posterior)	Boswell and Mahowald (1985); Schüpbach and Wieschaus (1986); Golumbeski et al. (1991)
<i>tudor (tud)</i>	57C7-9	6		mei	Hawley (1989)
<i>mei-W68</i>	2-94	2			

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Table 3 (Continued)

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>cricklet</i> (<i>clt</i>)	2-96	1		ooc	Shirras and Bownes (1989)
<i>torpedo (top)</i>	57F1	10	S	vent, lethal; EGF receptor mei	Clifford and Schüpbach (1989) Lindsley and Zimm (1990); Miyazaki et al. (1991)
<i>mei-S332</i>	58B1-C5	9		var, da	Schüpbach and Weischaus (1991)
<i>Wi42</i>	100	2		tum, degen	Schüpbach and Weischaus (1991)
<i>stall (stl)</i>	102	4		mei	Carpenter (1973); Miyazaki and Orr-Weaver (1992)
<i>ord</i>	59D4-10	1		degen; blocks oocyte determination	Mohler and Wieschaus (1986); Schüpbach and Weischaus (1991)
<i>egalitarian</i> (<i>egl</i>)	59F-60A	5		degen, nc number; also ms	Schüpbach and Weischaus (1991)
<i>shut down</i> (<i>shu</i>)	58D8-60A2	3		tum, small; also ms	Schüpbach and Weischaus (1991)
<i>peppercorn</i> (<i>ppc</i>)	59D8-60A2	2		cup	Schüpbach and Weischaus (1991)
<i>quit (qui)</i>	59D8-60A7	3		dump	Lindsley and Zimm (1990); Schüpbach and Weischaus (1991)
<i>minus (mi)</i>	59E1	2		degen (S4), sl	King (1970)
<i>morula (mr)</i>	106.7			tum; also ms	Gateff (1982); Gateff and Mechler (1989); G. Wei and A.P. Mahowald (pers. comm.)
<i>benign gonial</i>	60A3-7				Kimble et al. (1989, 1990)
<i>cell neoplasm (bgcn)</i>	60C	5	S?	cor, lethal	
<i>β3-tubulin</i> (β Tub60C)	60C	4	S?	MAT; blocks border cell migration; C/EBP	Montell et al. (1992)
<i>slow border</i>					
<i>cells (slobo)</i>					

^aSee Lindsley and Zimm (1985, 1990).^bMinimum number of alleles.^cAutonomous to S (soma) or G (germ line). ? indicates inferred from expression only.^dPhenotypic abbreviations: col (collapsed); cor (defective chorion; flaccid); cup (cup-shaped); da (defective dorsal appendages); degen (degenerating); dors (dorsalized); dump (dumpling); GV (germinal vesicle); MAT (maternal effect); ms (male sterile); mei (meiotic defects); ooc (defective oocyte); rud (rudimentary); S (stage); small (small egg); sl (semi-lethal); thin (thin chorion); tum (tumorous); var (variable); vent (ventralized).

Table 4 Third Chromosome Genes Involved in Oogenesis

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>tiny ovaries (tov)</i>	unmapped	5		rud	Tearle and Nüsslein-Volhard (1987)
<i>bric-a-brac (bab)</i>	61F	1	S?	rud or small ovary	Godt et al. (1992)
<i>rhomboid (rho)</i>	61F62D	2	S?	dors/vent	Mayer and Nüsslein-Volhard (1988); Ruhola-Baker et al. (1992)
<i>bipolar</i>	3-10	1		dicephalic	Tearle and Nüsslein-Volhard (1987)
<i>oocyte (bip)</i>					
<i>unicorn (uni)</i>	63D	2		dors/vent, dicephalic	R. Kelly (pers. comm.)
<i>encore (enc)</i>	63D/E	1		dors/vent, dicephalic; nurse cell number	Hawkins and Schüpbach (1992)
<i>Bf1</i>	64F	0		chromatin protein; enriched in GV	Frasch (1991)
<i>nudel (ndl)</i>	3-17	7	S	cor, MAT (dors)	Anderson and Nüsslein-Volhard (1984)
<i>spindle-C (spn-C)</i>	3-17	3		dors/vent, cor, dicephalic	Tearle and Nüsslein-Volhard (1987); Lindsley and Zimm (1990)
<i>s18, s15, s19, s16</i>	66D11-15	0	S?	chorion proteins	Spadling (1981); Orr-Weaver (1991)
<i>Tuba67C</i>	67C4-6	G?		α -tubulin synthesized only in nc	Theurkauf et al. (1986); Matthews et al. (1989)
<i>stonewall (stwl)</i>	70C/D	7		rud, tum	Berg and Spradling (1991); D. McKearin (pers. comm.)
<i>gonadal</i>	71CD	0			Schultz and Butler (1989); Schultz et al. (1990)
<i>neo31</i>	72C	1		thin; amplification normal	Cooley et al. (1988); R. Kelley (pers. comm.)
<i>transformer (tra)</i>	73A8-A9	6	S	visible, somatic sex	Belote et al. (1989)
<i>2877</i>	75C5-7	1		cup, elongated	C. Berg and A. Spradling (pers. comm.)
<i>dicaphalic (dic)</i>	3-46	(1)			Lohs-Schärdlin (1982); Frey and Gutzeit (1986); Bohrmann et al. (1986a)
<i>su(par)3-3</i>	3-46.6	25		degen	Szabad et al. (1988)
<i>fs(3)272</i>	3-47	1		thin, reduced amplification	Orret et al. (1984); Kelly and Spradling (1986)

(continued on following page)

Table 4 (Continued)

Gene ^a	Site ^b	Alleles ^c	Aut. ^d	Information ^e	References
<i>embryonic gonad</i> (<i>egon</i>)	79B	0		hormone receptor superfamily; Zn-finger-like knirps	Rothe et al. (1989)
<i>pipe</i>	3-47	9	S	ooc, MAT, dors	Anderson and Nüsslein-Volhard (1984); Letsou et al. (1991)
<i>ST2</i>	82E3	0		ovarian Zn-finger DNA-binding protein	Frank et al. (1990); J. Tower and A. Spradling (pers. comm.)
<i>kugeli</i> (<i>kug</i>)	3-47	10		small	Tearle and Nüsslein-Volhard (1987)
<i>doublesex</i> (<i>dsx</i>)	84E1-E2	3		visible, somatic sex	Burts and Baker (1989)
<i>oskar</i> (<i>osk</i>)	85B	9		ooc, MAT (posterior)	Lehmann and Nüsslein-Volhard (1986); Ephrussi et al. (1991); Kim-Ha et al. (1991)
<i>ovariette</i> (<i>ovt</i>)	85D	1		rud or small ovary	Lin and Spradling (1992)
<i>Dras1</i>	85D1	3	S	vent	C. Berg (pers. comm.)
<i>fragile chorion</i> (<i>fsh</i>)	3-55	2	S	cor	Tearle and Nüsslein-Volhard (1987); Stevens et al. (1990)
<i>6284</i>	86E3,4	1		degen (S8/9)	L. Schneider and A. Spradling (pers. comm.)
<i>nurse cell</i>	3-68	2		tum, degen, nurse cell number	Tearle and Nüsslein-Volhard (1987); Lindsley and Zimm (1990)
<i>number-1</i> (<i>ncn-1</i>)					Reuter et al. (1990)
<i>Suvar(3)7</i>	87E	1		variable; Zn finger protein, interacts with suvar(2)1 and suvar(3)3	Kelley (1990)
<i>squid</i> (<i>sqd</i>)	87F	5		dors	Tearle and Nüsslein-Volhard (1987)
<i>spindle-B</i> (<i>spn-B</i>)	88A10-C3	3		dors/vent, cor	Szabad and Bryant (1982); Orr et al. (1984); Kelley and Spradling (1986)
<i>fs(3)293, K43</i>	88B	25		thin, lethal; reduced amplification	King (1970); Parkhurst et al. (1988); Spana et al. (1988)
<i>su(Hw)</i>	88B	8	G?	degen	
<i>grapes</i> (<i>grp</i>)	88D	1		nurse cell number	R. Kelly (pers. comm.)
<i>spindle-E</i> (<i>spn-E</i>)	3-62	2		dors, vent, cor	Tearle and Nüsslein-Volhard (1987)
<i>c(3)G</i>	89A3	5		mei; recombination reduced	Lindsley and Zimm (1985); Szauder (1990)
<i>nanos</i> (<i>nos</i>)	91F-92A	5	G	α , MAT (posterior)	Sander and Lehmann (1988); Wang and Lehmann (1991); Lehmann and Nüsslein-Volhard (1991)
<i>Delta</i> (<i>Dl</i>)	92A2	>50		lethal; chambers fail to bud	Ruohola et al. (1991)
<i>bullywinkle</i>	92D1,2	2		abnormal anterior chorion	C. Berg and A. Spradling (pers. comm.)
<i>spindle-D</i>	3-91	2		dors/vent, cor	Tearle and Nüsslein-Volhard (1987)
<i>(spn-D)</i>					
<i>torsolike</i> (<i>tsl</i>)	93F6-14	7	S	ooc, MAT (terminal)	Sprenger et al. (1989); Stevens et al. (1990); D. Montell and A. Spradling (pers. comm.)
<i>spindle-A</i> (<i>spn-A</i>)	3-96	4		dors/vent, cor	Tearle and Nüsslein-Volhard (1987)
<i>orb-oo18-balb</i>	94F	3	G?	tum	Lantz et al. (1992); D. McKearin and A. Spradling (pers. comm.)
<i>twin</i> (<i>twn</i>)	95E8-F1,2	2		var; some 2 oocytes	Berg and Spradling (1991, pers. comm.)
<i>bag-of-marbles</i>	96C	4	G?	tum, cystoblast diff. blocked	McKearin and Spradling (1990)
<i>(bam)</i>					
<i>tiny eggs</i> (<i>tny</i>)	97D9-15	4		small	Tearle and Nüsslein-Volhard (1987)
<i>non-claret</i>	99C	>4	G?	mei	Sequeira et al. (1989); Yamamoto et al. (1989); Endow et al. (1990); Walker et al. (1990)
<i>disjunctional</i> (<i>ncd</i>)					

^{a,b}See Lindsley and Zimm (1985, 1990).^cMinimum number of alleles.^dAutonomous to S (sona) or G (germ line). ? indicates based on expression data only.^ePhenotypic abbreviations: col (collapsed); cor (defective chorion; flacid); cup (cup-shaped); da (defective dorsal appendages); degen (degenerating); dors (dorsalized); dump (dumpless); GV (germinal vesicle); MAT (maternal effect); ms (male sterile); met (meiotic defects); ooc (defective oocyte); rud (rudimentary); S (stage); small (small egg); sl (semi-lethal); thin (thin chorion); tum (tumorous); var (variable); vent (ventralized).

(*Bic-D, chickadee*), finding a sterile allele of a lethal gene is not generally thought to imply an important role for the gene in oogenesis. Sterility may be due to generally weakened viability or to pleiotropic effects that do not reflect any specific role in oogenesis, for example, alterations in protein synthesis. The phenotypic effects of lethal hypomorphs on egg development seem to fall into the same general classes as true steriles.

Many vital genes that are expressed in the ovary probably do not mutate readily to sterility due to a stringent requirement at earlier stages of development. Consequently, additional methods are needed to identify this class of genes. Rarely, isolation of a temperature-sensitive allele or ovary transplantation might indicate an oogenetic function. Pole cell transplantation provides a way to produce egg chambers with a mutant germ line, although the reciprocal constructs are impossible in the case of lethals (van Deusen 1976; Niki 1986). The dominant female sterile technique (Wieschaus 1980; Garcia-Bellido and Robbins 1983; Perrimon and Gans 1983) can provide an easier way to generate mosaic egg chambers if a suitable dominant female sterile mutation is available. The *ovo^{D1}* allele has been widely used to study X-linked genes because it arrests oogenesis relatively early and is not leaky (Perrimon et al. 1989a). Equally suitable mutations are not available for the other four major chromosome arms, although mosaics have been generated on 2L using *Fs(2)1* (Szabad et al. 1987, 1988; Macdonald and Struhl 1986). Because extensive searches have been tried (Table 1), the prospect of obtaining better dominant mutations (i.e., those producing rudimentary ovaries) seems to be low. Cloning of the *ovo* (4C) locus (Mevel-Ninio et al. 1991) may soon allow construction of strains with transformed copies of *ovo^{D1}* on other chromosome arms.

The dominant sterile technique has some limitations for studies of oogenesis. Unmarked clones can only be recognized if the mosaic egg chambers develop to a much later oogenetic stage than the large background of young egg chambers that remain heterozygous for *ovo^{D1}*. Suitable markers are lacking in the case of follicle cells. Furthermore, it may turn out to be difficult to generate a useful frequency of large mosaics within the follicle cells by normal somatic recombination, since multiple stem cells contribute to the follicle cells surrounding each chamber. Relatively small clones have proved useful when they effect the specialized anterior chorion (Stevens et al. 1990) or revert somatic-dependent dominant sterility (Szabad and Hoffman 1989). Fortunately, it may soon be possible to obtain more detailed information by using the high-frequency recombination catalyzed by the yeast *FLP* gene at its *FRT* target sequences (Golic and Lindquist 1989; Golic 1991; T. Xu and G.M. Rubin, unpubl.). If combined with a suitable marker system, labeled clones might be generated in a useful fraction of the egg chambers of each adult examined. The high frequency of clone generation might more readily yield large follicle cell mosaics.

Testing the phenotype of large numbers of lethals in germ-line clones by the dominant female sterile technique has provided the best information on the number of vital genes required for oogenesis (Perrimon et al. 1984, 1989a; Wieschaus and Noell 1986; Szabad et al. 1991). About 10% of the previously characterized X-linked lethal complementation groups studied by Perrimon et al. (1989a) produced specific abnormalities in oogenesis, although 50% of these caused only collapsed eggs. For example, germ-line mosaic egg chambers bearing mutants in *armadillo* (2B) produced small eggs, whereas mosaics mutant in the *Pgd* locus developed with a reduced number of nurse cells, and

chambers bearing the *DA689* allele of the *B-214* gene (Perrimon et al. 1989b) produced ventralized eggs. These results underestimate the true number of lethal genes that function during oogenesis, since lethals required in the germarium or during early stages could not be distinguished from the large number of cell lethals for which no clones were recovered. Unlike the true female steriles, the great majority of lethals that are important for oogenesis have not yet been identified and so are absent from Tables 2 through 4.

3. Visible Mutations

A second class of pleiotropic steriles are those with nonvital but morphological effects outside the ovary (Perrimon et al. 1986). These would include morphological mutations such as *rotund*, *rotated abdomen*, *cramped*, *flipper* (31B), *narrow* (2-83), and *stubarista* (12A) mutants with delayed development such as *tiny* and *diminutive* (3C), as well as probable physiological disruptions such as those caused by *cinnamon* and *lozenge*. Genes in this were easily identified and comprise many of the earliest female sterile mutations. Few genes in this small class have been shown to play a key role in oogenesis, however (see Tables 2 through 4).

B. Molecular Methods

Patterns of expression during oogenesis provide an alternative method of identifying candidate genes. Several potentially interesting genes have been studied due to their localized RNA (Ambrosio and Schedl 1984) or ovarian expression (Ait-Ahmed et al. 1992; Vincent et al. 1988; Schultz and Butler 1989). Improved methods of differential cDNA library construction should greatly enhance the sensitivity of this approach. Rapid whole-mount *in situ* hybridization techniques applicable to developing egg chambers (Tautz and Pfeife 1989; Ephrussi et al. 1991) allow a wider range of patterns to be readily recognized.

Enhancer-trap constructs allow tissue-specific and spatial patterns of hybrid gene expression to be visualized by simple histochemical stains for β -galactosidase (O'Kane and Gehring 1987; for review, see Wilson et al. 1990). Numerous enhancer-trap lines can be produced by mobilizing integrated transposons (see Table 1 and Figs. 4 and 5). About 50% of insertions (from male germ-line transpositions) are expressed somewhere in the ovary, although many share a few common expression patterns (Fasano and Kerridge 1988; Grossniklaus et al. 1989). The expression patterns observed in such lines have revealed previously unrecognized subgroups of follicle cells and can serve as useful cell markers (see Figs. 4 and 6). One limitation of enhancer-trap lines is their imperfect correspondence to endogenous patterns of gene expression (Wilson et al. 1990). However, defining lethal genes with important functions in oogenesis should be greatly assisted by selecting candidate genes for clonal analysis from collections of lethal single-*P* insertions with ovarian enhancer expression.

C. Developmental Methods

Developmental methods are extremely valuable in analyzing mutant phenotypes. For example, rescue experiments can assist in identifying key genes within genetic pathways (Anderson and Nüsslein-Vollhard 1984; Sander and Lehmann 1988). Unlike embryos, egg chambers cannot develop independently

of a host female, except during the last few hours (stages 10B to 14) of oogenesis (Srdic and Jacobs-Lorena 1978; Petri et al. 1979). However, egg chambers and even germaria can be isolated and maintained in culture medium for up to 1 hour and still develop to maturity if they are injected into the abdomen of host females bearing mutations such as *ovo^D*, which prevent extensive growth of the host's own ovaries (Gutzeit and Koppa 1982; Montell et al. 1991; Lin and Spradling 1992).

The *in vivo* culture approach allowed Montell et al. (1991, 1992) to study the developmental roles played by particular cells within stage-6–9 egg chambers. Ablating the germinal vesicle during this period with a laser microbeam ventralized the egg chamber and disrupted *vasa* localization, whereas elimination of the border cells helped reveal their specialized role in micropyle construction. Successful attempts to rescue mutant egg chambers by microinjection have not been reported. Furthermore, the inability to fertilize egg chambers that have developed to maturity in host females remains a major obstacle to learning the effects of treatments on embryonic development. In addition to enhancing the value of ablation and microinjection experiments, *in vitro* fertilization of mutants that hold apparently normal eggs within the ovary might reveal defects in their maternal components. Although unfertilized eggs have been activated *in vitro* (Mahowald et al. 1983), *in vitro* fertilization has never been accomplished in *Drosophila*, although it is successful in honeybees.

IV. MAJOR EVENTS OF OOGENESIS

A. Ovary Development

The *Drosophila* ovary arises from three separate sources during embryonic development (see King 1970; Mahowald and Kambyrellis 1980). Shortly after budding from the posterior end of the egg, the pole cells are carried dorsally by the elongating germ band, where they enter the posterior midgut rudiment. Moving between the endodermal cells, 30–40 pole cells split into two groups, migrate posteriorly, and associate with somatic mesodermal cells on each side of the embryo. About eight germ-line cells can be observed in 14-hour gonads, along with 10–20 somatic cells. These mesodermal cells are thought to derive from the fifth abdominal segment (A5) (see also Bate, this volume). The oviducts, accessory glands, uterus, vagina, and external genitalia of the adult ovary are derived from the genital imaginal disc and do not connect to the ovary until day 3 of pupation.

Before all of the parts of the adult ovary have even been assembled, differentiation of the ovary proper begins late in the third instar (see King 1970). During most of larval life, only a simple anterior-posterior organization is evident. The larger germ cells remain confined to the middle region of the ovary (see Lasko and Ashburner 1990), with smaller somatic cells at the poles (see Brower et al. 1981; Patel et al. 1987). The first morphological indication of differentiation is the stacking of subsets of the anterior somatic cells into 16 clusters of about ten disc-shaped cells. These cells will form the terminal filaments at the anterior end of each ovariole. Germ cells underlying each terminal filament are subsequently incorporated into a developing ovariole bounded by a distinctive extracellular matrix called the tunica propria. The

apical regions of all the ovarioles become attached to the peritoneal sheath at the top of the ovary and also near the posterior end. The somatic cells at the posterior presumably give rise to the basal stalk region of the ovary, i.e., cells involved in joining with the oviduct, and a lysosome-rich subset of cells postulated to function in recycling follicle cells sloughed off from mature egg chambers.

Germ cells are not required for early steps in ovary development. Despite a lack of pole cells, rudimentary ovaries develop in the progeny of grandchildless mutants (Fielding 1967; Niki and Okada 1981), the offspring of crosses between males from a strong P strain and M females (Engels and Preston 1979), in flies whose pole cells were destroyed experimentally (see Mahowald and Kambyrellis 1980), or by mutations such as *agametic* (Engstrom et al. 1982) or *ovo* (Oliver et al. 1987). Terminal filaments appear to develop despite the lack of germ cells. However, after a few days in adults, these may degenerate, and ovaries produced by some of these methods have other abnormalities, such as proliferating apical follicle cells that degenerate basally and excessively thick laminar membranes surrounding the ovarioles (see Engstrom et al. 1982). The "rudimentary" ovaries produced by most early mutants have not been studied in sufficient detail to determine whether they contain phenotypic defects distinct from a simple lack of germ cells.

B. Egg Chamber Formation

The germarium is an assembly line in which new egg chambers are produced from the progeny of germ-line and somatic stem cells (Fig. 4). Arrayed within a typical germarium are 12 cysts beyond the germ-line stem cells: 1 cystoblast, 3 successive stages of cyst formation, 6 cysts of 16 cells, and finally, at the posterior end, a single stage-1 egg chamber. However, the number of cysts varies substantially, depending on age and environmental conditions. All along the germarium, cysts move toward the posterior, due perhaps to pressure from dividing cells at the anterior tip or to the contraction of the sheath musculature. Cysts in the anterior half of the germarium are too small to span the diameter of the ovariole; occasionally, cysts move out of developmental order in this region (see Mahowald and Kambyrellis 1980; Carpenter 1981). In an adult (at 23°C), it takes about 7 days to proceed from a stem cell to a stage-2 egg chamber that has left the germarium (Wieschaus and Szabd 1979). However, the first cysts (formed in the pupal ovary) develop significantly faster than cysts produced under steady-state conditions (see Carpenter 1981; Grell and Generoso 1982). This suggests that the lineup of 16-cell cysts jamming a typical adult germarium could develop more rapidly were traffic lighter. Individual germarial cysts cannot generally be recognized in the light microscope, although staining with anti-*vasa* sera highlights later stages. Thus, knowledge has been primarily based on sectioned material, including a very small number of germaria reconstructed from serial electron microscope sections.

1. Stem Cells

Visual identification of approximately two germ-line stem cells at the tip of each ovariole was confirmed by clonal analysis (Schüpbach et al. 1978; Wieschaus and Szabd 1979). Furthermore, these studies revealed several other facts about ovarian stem cells. Stem cells usually (perhaps invariably) divide

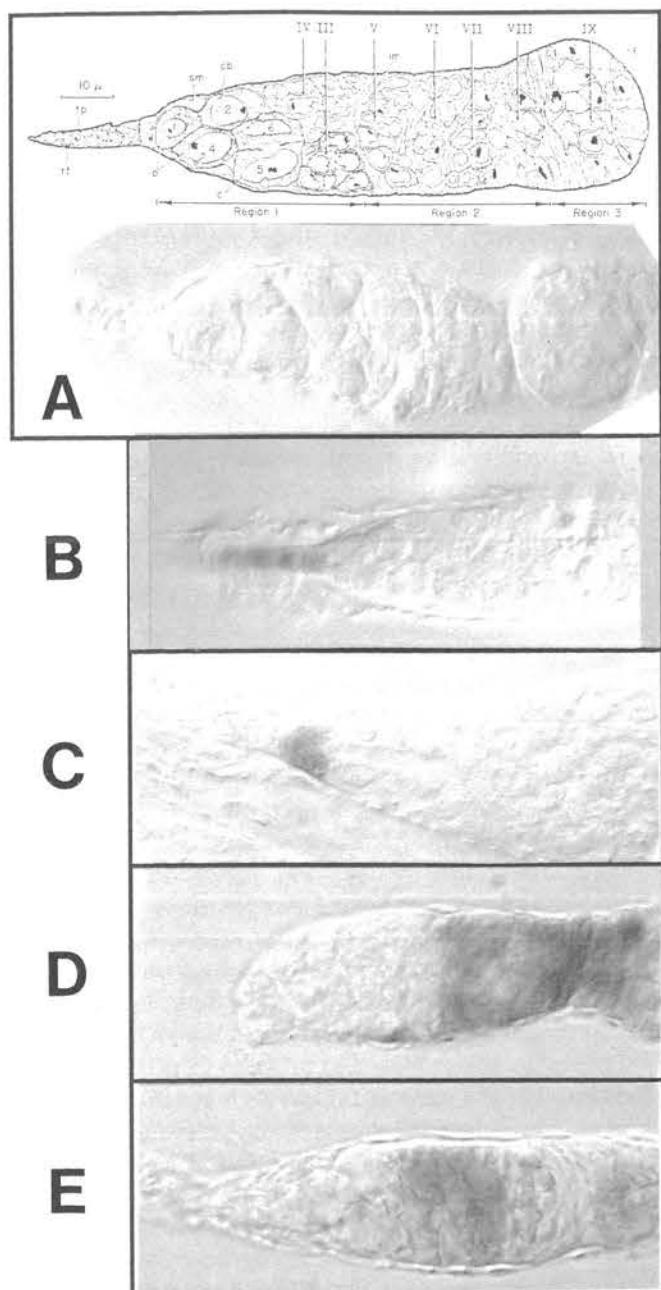


Figure 4 Germarium structure and enhancer-trap expression patterns. In *A*, a drawing (reprinted, with permission, from King 1970) of a cross section of a germarium is shown above a Nomarski photograph of a similar germarium. The terminal filament (tf), stem cells (*o*), cystoblasts (*c, cb*), follicle cells (*sm, im, cf*), and basement membrane (*tp*) are indicated; also shown are 7 cysts of 16 cells (III-IX), and the three major regions of the germarium. (*B-E*) Germaria from enhancer-trap lines that were fixed and stained with X-gal. Labeled cells: (*B*) terminal filament; (*C*) three somatic cells near stem cells; (*D*) migrating follicle cells; (*E*) germ-line cyst cells in regions 2 and 3. Magnification, 750 \times .

asymmetrically to produce a daughter stem cell and a cystoblast. The distribution of clonally marked eggs showed that the two to three stem cells do not alternate regularly, but undergo bursts of two to five divisions in succession before another stem cell takes over. Ablation of the stem-cell region at the tip of the germarium did not prevent surviving germ-line cells from developing into mature eggs; however, no new cysts formed (Lin and Spradling 1992).

Terminal filament cells (Fig. 4B) lie next to the stem cells and may regulate stem cell proliferation in a manner similar to that of the distal tip cell of the nematode gonad (see Austin and Kimble 1987). Contact with a terminal filament cell might inhibit stem cell differentiation into cystoblasts. This could explain why only the two to three cells at the very tip of each ovariole are maintained as germ-line stem cells. Alternatively, signaling between terminal filament and stem cells might regulate the timing of stem cell divisions. It is not clear from these models why ten terminal filament cells should be needed or why they are stacked in such a way that only the posterior cells are close to the stem cells. Several enhancer-trap lines specifically expressed in the terminal filament have been recovered (Fig. 4B). Another set of about three somatic cells that may play a role in stem cell regulation are asymmetrically positioned near the stem cells (Fig. 4C). Some enhancer-trap lines specifically label the terminal filament and these cells. A subset of sheath cells that overlies the terminal filament forms a third specialized group of cells near germarial tips. Several candidate genes have been identified by mutation that may function in maintaining stem cells (*bric-à-brac* [61F], Gott et al. 1992; *ovarette* [85D], *piwi* [32C], Lin and Spradling 1992).

In addition to the terminal filaments, at least 100 other somatically derived cells reside near the outer surface of each germarium. Clonal analysis using the *dec-1* chorion mutation as a marker (Wieschaus et al. 1981) suggests that about 20 precursor cells to all adult follicle cells exist in the second instar gonad. However, little is known about the subsequent cell lineages and differentiation of germarial somatic cells. Stem cells must be present to replenish the follicle and interfollicular cells lost as each new egg chamber leaves the germarium. No definitive evidence regarding these stem cells is available, but they may reside about halfway down the germarium, surrounding the region where prefollicle cells migrate inward to envelop germ-line cysts. Electron microscopy autoradiographic studies detected labeling of follicle cells with [³H]thymidine no farther anterior than this region (Carpenter 1981). Follicle stem cells would be expected to lie in two rings: a larger anterior ring that generates prefollicle cells and a posterior ring whose products might produce the interfollicular cells that separate adjacent cysts.

2. Cyst Formation

Each new egg chamber begins development in the anterior portion of the germarium (region 1, see Fig. 4) through the production of a 16-cell germ-line cyst. Cyst formation initiates when one of the stem cells at the tip of the germarium divides unequally to produce a cystoblast. The new cystoblast immediately commences a pattern of cell division very different from that of its sister stem cell. It divides exactly four more times, but the process of cytokinesis is now altered so that daughter cystocytes remain interconnected by specialized cleavage furrows called ring canals. The four cystocyte divisions are cleavage-like; daughter cells remain smaller than the parent cysto-

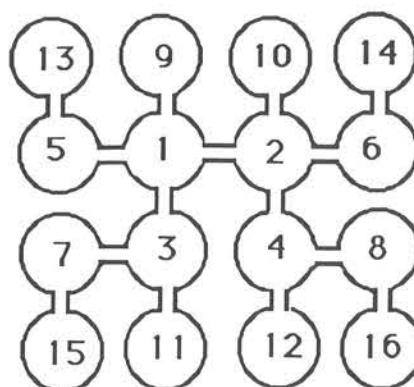


Figure 5 Pattern of interconnections within a 16-cell cyst. The interconnections produced by the four cystocyte divisions are shown diagrammatically, according to the standard nomenclature (King 1970). One of the two cells with four ring canals, i.e., cell 1 or cell 2, always becomes the oocyte in a normal egg chamber.

blast ($50 \mu\text{m}^3$ vs. $200 \mu\text{m}^3$ according to King [1970]), indicating that division was triggered prior to a doubling of cell mass. Cystocytes divide synchronously, possibly because their cytoplasms remain interconnected by ring canals. They also divide rapidly; labeling studies and the presence of only one to three cysts with less than 16 cells per ovariole suggest that only 24 hours may be required for a cystoblast to complete cyst formation.

The lineage of individual cystocytes can be partially determined from the number of ring canals that remains in each cell. The first two cells retain four canals, the next two retain three canals, the next four retain two canals, and the remaining eight cells have just one canal (Fig. 5). In older egg chambers, the number of ring canals can be determined by immunofluorescent staining with rhodamine-conjugated phalloidin (Warn et al. 1985). In germarial cysts, serial section electron microscopy has allowed reconstruction of the pattern of interconnections.

Completion of the cystocyte divisions marks another major cell cycle change. All 16 cyst cells subsequently enter a long S phase that constitutes the premeiotic S phase of the future oocyte. As in other organisms, premeiotic S phase is extended compared to the preceding cell cycle. Autoradiography experiments suggest that euchromatin is replicated first, followed after a period of overlap by labeling only within heterochromatin (Carpenter 1981). As development progresses, the synchrony of the 16 cyst cells is lost. Only one cell continues through meiosis and becomes the oocyte, and the other 15 begin to grow and differentiate as nurse cells.

Nurse cells diverge sharply from the developmental pathway followed by the oocyte. Initially, multiple cystocytes assemble synaptonemal complexes, strongly suggesting that some future nurse cells, like the oocyte, enter meiosis. In some insects, all cystocytes initially form a synaptonemal complex. Although their fate may transiently be ambiguous, by the time they exit the germarium, all 15 nurse cells have left the meiotic cycle. They begin to grow rapidly without further division, even forming polytene chromosomes in many species (Ribbert 1979; Redfern 1981). Changes in the nurse cell genome occur during polytenization, such as the differential loss of satellite DNAs and

inserted rDNA. However, these changes are less extensive than those in many other somatic cells (see Spradling and Orr-Weaver 1987; Spradling et al. 1992).

3. Cyst Envelopment

The large middle portion of the germarium that contains 16-cell cysts is termed region 2 (Fig. 4). As they move posteriorly, cysts become surrounded by a layer of follicle cells. However, envelopment is a slow process; cysts in the anterior portion known as region 2a have not been fully enclosed by follicle cells and still directly contact neighboring cysts. Subsequently, in region 2b, inwardly migrating follicle cells separate off individual cysts. At this point, characteristic lens-shaped cysts are observed that now span the entire distance between the walls of the germarium. Their shape becomes rounder as they near the end of region 2, after which inwardly migrating pre-interfollicular stalk cells separate out the stage-1 egg chamber. Before separation is complete, stage-1 chambers are considered to constitute region 3 of the germarium.

Follicle cells migrate from the wall of the germarium to surround germinal cysts, the first of several major migrations these cells accomplish during oogenesis (Fig. 4D). Follicle cells do not associate with 8-cell cysts, but their cellular processes contact 16-cell cysts shortly after the fourth cystocyte division. The affinity of region-2 follicle cells and 16-cell cysts may result from the induction of a specific ligand on the cystocyte surface or the expression of a follicle cell receptor for an existing cystocyte ligand. The number of follicle cells that initially surround a cyst is difficult to determine, since the chambers are closely packed and some germarial somatic cells will contribute to the interfollicular stalk. Carpenter suggests that the initial number is about 16. King (1970) counted about 80 follicle cells surrounding stage-1 egg chambers, so one to two follicle cell divisions may occur in the germarium following association with a cyst.

Several genes appear to function in enveloping cysts with follicle cells and budding chambers off of the germarium. The proliferating germ-line cells in "ovarian tumor" mutations often are not surrounded by follicle cells. This is not surprising if 16-cell cyst formation is required to induce follicle cell binding. However, weaker alleles at many of these loci allow egg chambers with an excess number of germ-line cells to bud off, suggesting that the association of germ-line and somatic cells into egg chambers may be more complex. At least two neurogenic genes, *Notch* and *Delta*, encoding membrane proteins with EGF-like repeats, appear to be required in follicle cells for egg chamber formation (Ruohola et al. 1991; Xu et al. 1992). The neurogenic locus *brainiac* (3F) is required in the germ line, and cooperates with the *Drosophila* EGF receptor (*torpedo*), for the inward migration of germarial follicle cells (Goode et al. 1992). A second chromosome female sterile mutation (*fs(2)LYH*) may define another function in cyst envelopment. Homozygous females contain ovarioles lined with follicle cells in which cysts develop to late stages without ever pinching off into separate egg chambers (L. Yue and A. Spradling, pers. comm.).

4. Oocyte Determination and Differentiation

In the polytrophic ovaries of insects such as Dytiscid water beetles, all the cystocyte divisions are visibly unequal, revealing that the initial cell (cystoblast) always becomes the oocyte (see Gall et al. 1969). A similar situation is likely to hold in *Drosophila*, since the oocyte always contains four ring

canals (Fig. 5). Almost nothing is known about processes of oocyte determination during cyst formation. Subsequently, oocytes diverge rather slowly from the other 15 cystocytes; they cannot be morphologically recognized in the electron microscope until many hours later, when cysts are midway through region 2 of the germarium. A variety of studies suggest that differential transport through the ring canals is necessary as cysts develop to effect oocyte differentiation.

Morphological studies revealed the first evidence for differential transport of materials from the future nurse cells to the oocyte (Mahowald and Strassheim 1970). During the cystocyte divisions, a complex cytoskeletal transformation leads to the production of an asymmetrical ring canal complex (or "polyfusone") spanning all 16 cells (see Storto and King 1989). Bulk flow of cytoplasmic constituents through ring canals is not apparent in early region 2 cysts, but a "nutrient stream" commences late in region 2a. Increases in ring canal diameter, the disappearance of spindle remnants, and development of extensive microtubule bundles within the canals all suggest that mechanisms to facilitate and regulate intercellular transport are organized in early cysts. The directional nature of movement through ring canals was first revealed by studies of the fate of the cystocyte centrioles. Some of the 32 centrioles and procentrioles present in the 16-cell cyst appear to degenerate, but many others move away from their normal location near the nuclear membrane, approach ring canals, and make their way specifically into the future oocyte.

Genetic studies provided evidence that differential transport might be important for oocyte formation. Several *Bic-D* mutations prevent oocyte formation, leading to cysts with 16 nurse cells. *Bic-D* RNA and protein are expressed in all cystocytes as cysts form but then being to accumulate selectively in the future oocyte as cysts traverse region 2 (Suter et al. 1989; Wharton and Struhl 1989; Suter and Steward 1991). *Bic-D* mutations that disrupt oocyte formation interfere with the enrichment of *Bic-D* RNA in the oocyte. In the R26 allele, however, *Bic-D* protein still accumulates selectively in one cell of each cyst, suggesting that other differential transport mechanisms may exist within cysts in addition to those that localize mRNAs. Transcripts from the posterior group genes *oskar* (Ephrussi et al. 1991; Kim-Ha et al. 1991) and *tudor* (57C) (Gulbenkesi et al. 1991), the RNA-binding protein gene *orb* (94F) (Lantz et al. 1992), the *BJ1* (64F) chromatin protein gene (Frasch 1991), and the *hu-li tai shao* (*hts*) (56C) gene encoding an adducin-like protein required for normal ring canal formation (Yue and Spradling 1992) also accumulate selectively in the oocytes of germarial cysts. Strong alleles of *hts* greatly reduce the frequency of oocyte formation, possibly because the abnormal ring canals interfere with intercellular transport. Thus, a cystocyte that is determined to become the oocyte under ordinary circumstances is likely to revert to a nurse cell fate without the transport and selective accumulation of multiple components produced throughout the cyst.

The ability of cysts to differentially transport mRNAs and other components into the oocyte is probably acquired by a major reorganization of cytoskeletal elements including microtubules (Theurkauf et al. 1992). By the time cysts reach region 3, a single microtubule-organizing center can be visualized in the oocyte that appears to organize microtubules extending throughout the cyst. Microtubule-based transport could propel the movement of centrioles and mitochondria through ring canals and explain why col-

chicine treatment frequently blocks oocyte differentiation (Koch and Spitzer 1983). This mechanism could also explain several peculiarities of cyst development. For example, oocyte-specific products in transit through adjacent cells might cause the transient appearance of synaptonemal complexes in the other 4-ring-canal cell, and in some 3-ring-canal cells.

Cysts interact extensively with follicle cells as they move through the posterior region of the germarium; however, there is no evidence that these interactions are important for oocyte differentiation. At this time, the oocyte normally comes to lie at the posterior of the cyst, one of the earliest signs of the anterior-posterior axis. How this polarity arises remains uncertain. The encircling follicle cells might convey spatial information, particularly the interfollicular stalk cells that lie both anterior and posterior of each stage-1 egg chamber. Mutations in the gene *dicephalic* (3-46), *spindleC* (3-17), *bipolar oocyte* (3-10), *unicorn* (63D), and *encore* (63D) disrupt this polarity. Oocytes in a minority of chambers produced by these mutations lie somewhere in the middle of the cyst, with the 15 nurse cells distributed variably both anteriorly and posteriorly. Oocyte determination per se is not affected, however, and these chambers complete oogenesis.

5. Regulation of Cyst Formation

An enigmatic class of mutations called ovarian tumor mutants disorganize the germaria and result in the production of abnormal egg chambers containing excessive numbers of cells (King 1970). Such mutations arise in the genes *fs(2)B*, *fused* (17C), *narrow*, *ovarian tumor*, *sans fille* (4F), *benign gonial neoplasm* (60A), *ovo*, *bag-of-marbles*, *stonewall*, and *orb* (see Tables 2-4). Furthermore, several lethal genes were found to produce similar defects, when present in homozygous germ-line clones (*Sex-lethal* [6F], Schüpbach 1985) or when homozygous mutant ovaries were transplanted into wild-type hosts (*lethal* (2) *giant larvae* [21A], Gloor 1943). Since genetic screens have not reached saturation, at least 20 genes appear to be capable of mutating to cause tumorous ovaries.

The normal function of ovarian tumor genes has been difficult to determine. The phenotypes produced by *otu* alleles (for review, see King and Storto 1988) or *ovo* alleles (see Oliver et al. 1990) range from nearly agametic ovaries to tumors, to abnormal degenerating chambers, to ovarioles that produce many late egg chambers showing various defects. Certain alleles regularly produce "tumors," in which ovarioles are filled with small dividing cells and egg chambers containing hundreds of small cells mixed with large polytene pseudonurse cells. Molecular studies revealed that probable *otu* null alleles, including many that damage or partially delete the coding region, produce the "quiescent" phenotype, i.e., rudimentary ovaries lacking evidence of germ cells (Steinhauer et al. 1989; Steinhauer and Kalfayan 1992). Strong *ovo* alleles also result in early female-specific pole cell death (Oliver et al. 1987). However, the tumorous state does appear to represent the null ovarian phenotype of at least two genes, *Sxl* (Schüpbach 1985; Steinmann-Zwicky et al. 1989) and *bam* (McKearin and Spradling 1990). Structural studies suggest that ovarian tumor genes carry out diverse biochemical functions. *otu* and *bam* encode novel proteins that share a region of weak similarity. At least in somatic cells, the proteins encoded by *Sxl* appear to bind RNA and regulate splicing. The *orb* gene encodes a putative RNA-binding protein (Lantz et al. 1992; D.

McKearin, unpubl.). The protein encoded by *lgl* is thought to function as a cell adhesion molecule (Klambt et al. 1989), whereas *fused* encodes a putative serine-threonine kinase (Preat et al. 1990).

The four cystocyte divisions that produce 16-cell germ-line cysts in both the male and female germ lines appear to be the target of at least two tumor genes, *bag-of-marbles* and *benign gonial neoplasm*. These genes may regulate a common step in male and female cyst formation; both sexes are rendered sterile by the mutants. *bam* alleles produce abnormal cysts with an excess number of cells in both sexes. Transcripts from the gene are expressed specifically in cystoblasts, suggesting that *bam* programs the number of cystocyte divisions. Storto and King (1989) have proposed that *otu* encodes a component of the fusome, without which cystocyte division becomes deranged. Null alleles would block further division, and tumorous alleles would circumvent normal regulation by the fusome. Homozygous *otu* egg chambers that develop almost to maturity before arresting were postulated to suffer from defective ring canals.

Recent studies have revealed a relationship between tumorous egg chambers and the process of sex determination. Sex determination in the germ line (for review, see Pauli and Mahowald 1990) is under a control very different from the relatively well-characterized pathway of somatic sexual differentiation. Most somatic sex determination genes are not required in either the male or female germ line (Schüpbach 1982; Cline 1983, 1984). Germ cells use both autonomous and nonautonomous mechanisms to decide their sexual identity (Nöthiger et al. 1989; Steinmann-Zwicky et al. 1989). Signals from the somatic mesodermal cells of the gonad appear to be necessary for germ cell differentiation, although the exact nature of the signaling, its timing, and the information transmitted remain to be determined. Tumorous cysts are produced if the germ cells lack *Sxl* (Schüpbach 1985), suggesting that germ-line sex determination is necessary for normal cystocyte divisions. It remains unclear how germ-line cells measure the X-autosome ratio and how or even whether they dosage-compensate X-linked genes.

At least three of the ovarian tumor genes appear to act by disrupting the pathways that establish or maintain germ-line sexual identity. In addition to *Sxl*, the *sans-fille* (or *liz*) gene mutates to produce ovarian tumors. Interactions of *snf* and *Sxl* argue that *snf* is required to maintain *Sxl* activity in both the soma and the female germ line (Oliver et al. 1988; Steinmann-Zwicky 1988). Oliver et al. (1990) used genetic interactions between *Sxl* and *ovo* to argue that *ovo* is also involved in germ-line sex determination. Perhaps the early requirement for *ovo* in female germ cells is due to a related function such as dosage compensation.

A role in germ-line sex determination might explain the great phenotypic variety associated with lesions in several ovarian tumor genes. Germ cells may have to communicate repeatedly with somatic ovarian cells to maintain their sexual identity. The tumor phenotype per se may result because germ-line cells must be female to begin differentiation as cystoblasts. Expression of the testis-specific product of the *Stellate* locus (Livak 1990) in small proliferating germarial tumor cells suggests that some of these cells become transformed toward primary spermatocytes. Defects in more mature egg chambers may result when sexual differentiation is initiated correctly but fails to be maintained at later stages of oogenesis. The hypothesis that germ-line cells

must communicate to maintain their determined state can also explain why a diverse group of genes might mutate to the tumorous state. Genes that disrupt signaling between germ line and soma by whatever mechanism would disrupt sex determination. Thus, genes controlling cell proliferation, germarium structure, or cell signaling might all mutate to a tumorous phenotype.

A second group of genes alters cyst cell numbers. *encore* and *grapes* (88D) homozygotes produce egg chambers with up to 31 nurse cells, suggesting that cystoblasts have been reprogrammed to divide five times rather than four. *hu li tai shao* egg chambers contain an average of only four to six nurse cells, with or without an oocyte (see Yue and Spradling 1992). Cytokinesis may proceed too far during cystocyte divisions in the mutant, completely separating groups of daughter cystocytes. Furthermore, the ring canals joining cells in these chambers remain structurally abnormal and contain reduced amounts of actin. The *hts* gene encodes a homolog of adducin, a vertebrate cytoskeletal protein that links actin to spectrin in red blood cell membranes. During oogenesis, *Drosophila* adducin may stabilize ring canals and participate in their growth and association with actin.

C. Meiosis

1. Recombination and Conversion

The cytological events of meiotic prophase have been difficult to establish (King 1970; Mahowald and Kambyrellis 1980; Carpenter 1981; Grell and Generoso 1982). Synaptonemal complexes have formed in third or fourth rank 16-cell cysts within region 2a. Reconstructions allow determinations of the three-dimensional organization of the major chromosome arms (Carpenter 1975). Synaptonemal complexes are found not only in the future oocyte, but also in the other four-ring canal cell and sometimes in cells with just three-ring canals. Exactly when meiotic prophase starts and when the pachytene stage (characterized by maximal synaptonemal complex length) is attained have remained controversial. King (1970) placed pachytene in stage 3, shortly before karyosome formation, whereas Carpenter (1975) reported that germarial cysts in region 2a reach pachytene. Another controversy concerns whether synaptonemal complexes form during interphase when DNA replication is ongoing. It seems more likely that their appearance and characteristic contraction in *Drosophila* correspond to the pachytene stages of meiotic prophase described in other organisms. Localization of cell-cycle-specific antigens within region 2 may help resolve when cysts carry out events of meiotic prophase. Specialized structures termed recombination nodules were observed by Carpenter beginning four to five cysts into region 2a. Considerable circumstantial evidence associates these structures with sites of meiotic recombination; however, there is no direct evidence for this conclusion, and it remains uncertain if they are comparable to recombination structures observed in other organisms (Carpenter 1979a,b). The inability to visualize meiotic chromosomes easily remains a limitation in analyzing the role played by meiotic genes. Only when the karyosome has begun to decondense early in stage 13 can chromosomes be visualized in the light microscope (Puro and Nokkula 1977).

Drosophila meiosis has long been a favorite subject for genetic studies (for reviews, see Baker and Hall 1976; Carpenter 1988; Hawley 1988). Sites of gene

conversion and recombination appear to be distributed more uniformly along the euchromatin in *Drosophila* than in organisms such as *Saccharomyces cerevisiae* or *Caenorhabditis elegans* (Clark et al. 1988; Curtis et al. 1989; Curtis and Bender 1991). In the *rosy* region, the frequency of conversion tracts did not vary more than about twofold along the gene. Direct sequencing revealed that conversion tracts averaged about 800 bp in length. Many mutations affecting meiosis have been described. *c(3)G* (89A) nearly abolishes recombination. The *cdc25* homolog *twine* (35F) functions exclusively during male and female meiosis (Courtot et al. 1992). Mutations that disrupt recombination, such as *mei-41* (14C) and *mei-9* (4C), probably encode ubiquitous DNA repair proteins (Gatti et al. 1981). However, *mei-218* may function specifically in recombination (see Carpenter 1982, 1989), although its null phenotype remains unknown.

P-element excision may provide a tractable system for studying specific gene conversion events in both meiotic and nonmeiotic cells. Excising *P* elements generate a gapped template that becomes a hot spot for site-specific conversion, using information donated by a sister, a homolog, or even an ectopic copy introduced by transformation (Engels et al. 1990). These events occur in both the male and female germ lines prior to and during meiosis. Somatic cells are probably also able to gene-convert gapped DNAs, since precise excisions occur in many tissues with high frequency (Laski et al. 1986).

2. Disjunction

Like other organisms, disjunction during meiosis I normally requires recombination to align the homologs on the spindle. However, *Drosophila* females also simultaneously utilize an alternative system of disjunction called distributive pairing. Chromosomes that do not regularly undergo exchange, such as the small fourth chromosomes, segregate efficiently via the distributive system. Rare tetrads that do not undergo crossing over also enter the distributive pathway. Most genes affecting disjunction probably do so indirectly by affecting recombination. However, two genes, *mei-S332* (58B) and *ord* (59D), appear to play a specific role in keeping sister chromatids together during meiosis I (Miyazaki et al. 1991; Kerrebrock et al. 1992).

Studies of two other genes required for disjunction, *claret-non disjunctional* (*cand*) (Endow et al. 1990) and *no distributive disjunction* (*nod*) (10C) (Zhang and Hawley 1990; Theurkauf and Hawley 1992), have shed light on the molecular mechanisms of meiotic chromosome segregation. *cand*, now known as *non-claret disjunctional* (*ncd*) (99C) (Sequeira et al. 1989), is required for efficient disjunction of the exchange and nonexchange homolog during meiosis I; it also exerts a maternal effect on chromosome disjunction during the first zygotic division. In contrast, *nod* specifically affects chromosomes segregating via the distributive system; disjunction of such chromosomes also remains defective during the first few zygotic mitoses. Both genes encode proteins that are structurally related to kinesins, i.e., proteins that act as microtubule-based motors. The Ncd protein encodes a novel kinesin that directs movement in a direction opposite to that of previously characterized kinesins (McDonald et al. 1990; Walker et al. 1990). The Ncd kinesin has properties that might allow it to draw chromosomes along spindle fibers during the process of disjunction. In contrast, Nod protein may exert a force toward the plate that somehow aids in distributive segregation. Furthermore, the observation that chromosomes

derived from mutant mothers are frequently lost during the first few cleavage divisions of a heterozygous embryo suggests that the defective proteins are associated with the maternal chromosomes (although other models remain possible). Presumably, the faulty proteins are not diluted out by wild-type products for several divisions after fertilization.

D. Egg Chamber Growth

1. Nurse Cell and Oocyte Development

Egg chambers spend about 2 days between the time they leave the germarium and the onset of vitellogenesis. This appears to be primarily a time of growth. However, the development of the oocyte and nurse cells continues to diverge (Fig. 6A). The oocyte nucleus transcribes actively in germarial cysts, but it becomes repressed shortly after egg chambers leave the germarium. Oocyte chromosomes condense into a "karyosome" by stage 3. The germinal vesicle remains considerably larger (Fig. 7) and develops a distinctive "endobody" similar to that observed in other Diptera (see Mahowald and Kambsellis 1980). Structures that may be related to the *Drosophila* endobody have been described recently in association with specific loops of newt lampbrush chromosomes and in oocyte nuclei from several diverse species (Gall and Callan 1989). These "sphere organelles" were postulated to play a role in assembling small nuclear ribonucleoprotein (snRNP) complexes.

Nurse cells also undergo changes in their nuclear organization during this growth period. Following the initial rounds of endoreplication, nurse cell chromosomes remain somatically paired. In some Dipteran nurse cells, large polytene chromosomes subsequently develop. In *Drosophila*, however, the association between homologs weakens after stage 4, and large-scale organization of nurse cell chromatin can no longer be seen. Satellite DNAs become underrepresented as nurse cell DNA content increases (Hammond and Laird 1985), but nurse cells retain more satellite DNA than other polyploid cells. Nurse cells also develop unusual nucleoli that by stage 9 comprise a shell of interconnected fibers around the periphery of the nucleus. Nurse cells retain more rRNA genes than other polyploid cells and synthesize rRNA at proportionately higher rates (see Spradling and Orr-Weaver 1987). Their nucleolar structure probably represents a further adaption to high levels of ribosome production. Spradling et al. (1992) compared nurse cell genomic alterations to those in developing macronuclei from hypotrichous ciliates and suggested that they may function to produce the modified nucleoli.

Mechanisms must exist to coordinate the growth of the nurse cells and oocyte, since their volumes increase at a similar rate despite the inactivity of the germinal vesicle. Cytoplasmic growth must be controlled in part by the rate of transport of materials through ring canals and into the oocyte. Cells located closer to the oocyte, particularly the four cells directly connected via ring canals, frequently undergo an extra replication cycle. Regulation of nurse cell growth is altered in mutants such as *dicephalic*, where some nurse cells are located on each side of the oocyte (Bohrmann et al. 1986a). Such chambers contain up to 45% more nurse cell DNA at stage 10. Nurse cell DNA content is inversely correlated with the number of nurse cells in each nurse chamber, regardless of its anterior or posterior location. These observations argue that nurse cell growth is regulated by signals passing between the oocyte and each

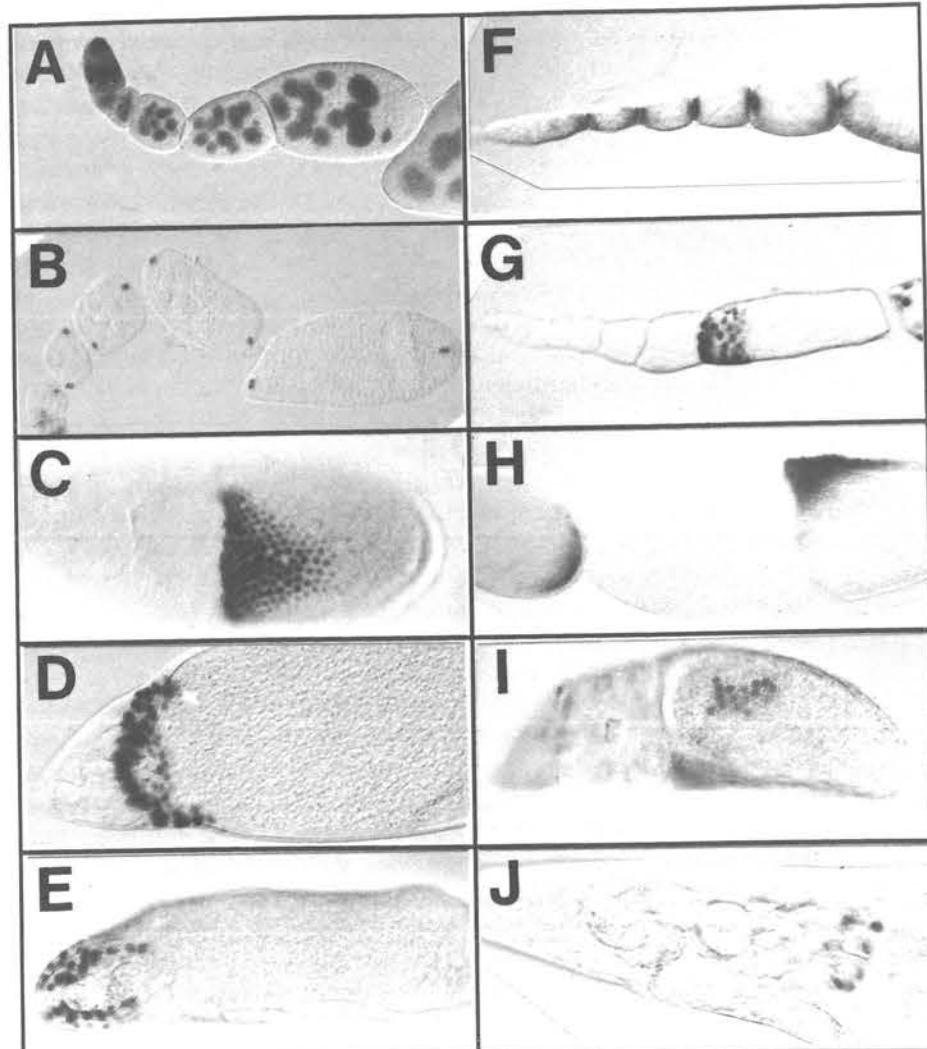


Figure 6 Common expression patterns in enhancer-trap lines. Ovaries were dissected, fixed, stained with X-gal, and photographed. (A) Germ-line-specific staining, all stages; (B) two polar follicle cells (like fasciclin III); (C) dorsal follicle cell patch at S10; (D) follicle cells forming the "collar" region of the operculum at stage 12; (E) follicle cells along dorsal appendages at stage 13; (F) interfollicle cells and some follicle cells; (G) follicle cells that remain over the nurse cells beginning at stage 9 (prior to migration); (H) cells that remain over the nurse cells beginning at stage 9 (prior to migration); (I) follicle cell posterior patch of follicle cells at stage 8, and dorsal patch at stage 10; (J) follicle cell band resolving into patch of cells where bases of dorsal appendages will form; (J) ring of border cells surrounding micropyle tip at S13. Magnifications: (A-D, F-I) 150x; (E) 112x; (J) 375x.

nurse cell cluster as a whole. Similar though smaller increases in nurse cell DNA content were observed in *spindleC* chambers (Bohrmann et al. 1986a).

Follicle cells initially divide to maintain a monolayer around the swelling nurse chamber. Four to five divisions are required to bring their number close to 1000 by the end of stage 5. Subsequently, the follicle cells also cease cell

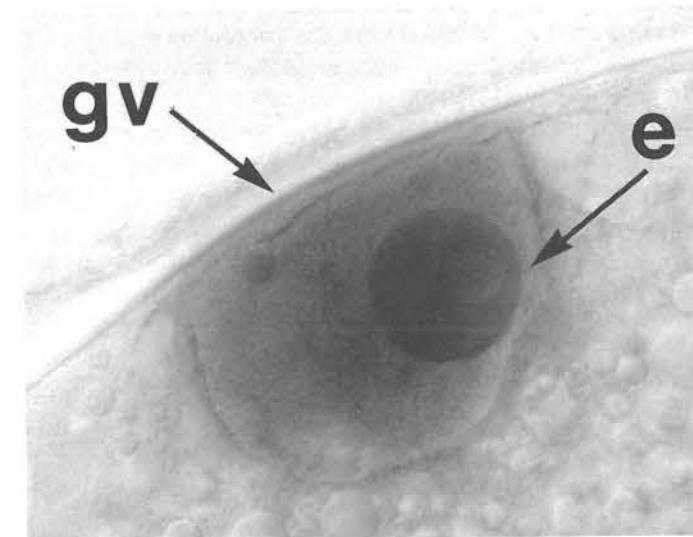


Figure 7 Germinal vesicle (gv) and endobody (e) from stage-10A egg chamber. Magnification, 1000x.

division but increase further in size by polypliodization. Despite some disagreement among four groups that have measured follicle cell DNA contents (Mahowald et al. 1979; Hammond and Laird 1985; Mulligan and Rasch 1985; Bohrman et al. 1986a), it appears that follicle cells undergo up to five rounds of endoreplication after stage 5. Satellite DNA becomes underrepresented, as in the case of other somatic polypliod or polytene cells (for review, see Spradling and Orr-Weaver 1987).

2. Regulation of Egg Chamber Differentiation

Several genes appear to be specifically required for the proper differentiation of cystocytes into nurse cells and an oocyte. *Egalitarian* and *Bic-D* mutations cause all 16 cyst cells to develop as morphological nurse cells (prior to degenerating). Many other early mutations affect nurse cells, leading to abnormal egg chambers that eventually degenerate. Egg chambers in such strains may contain more or less than 15 nurse cells (*hts*, *grapes*, *encore*, *ncn-1* [3-68]) and may exhibit abnormal nuclear morphology, such as highly condensed chromatin (H. Lin and A. Spradling, pers. comm.) or abnormally persistent polytene chromosomes (*su(Hw)*, *otu*, *fs(2)B*) (see Heino 1989). In some cases, egg chamber growth is arrested for a long period prior to degeneration, causing a line of similar-sized chambers to accumulate in each ovariole, a condition referred to as beads-on-a-string (*boas*). Some degenerating mutations are associated with semilethality and some are leaky, producing late-stage chambers that may exhibit characteristic pattern defects (*Bic-C* [35E], *cup*, *zeppelin* [47A]). At least 30 genes falling into this general class are known (Tables 2-4), and an equal number probably remain to be discovered.

One of the first genes to be studied that displays a degenerating phenotype was *suppressor of Hairy wing* (King 1970). *su(Hw)* encodes a zinc finger protein that binds to an enhancer within the retrovirus-like transposable element *gypsy* (Spana et al. 1988; Mazo et al. 1989). Binding is required for *gypsy*-

mediated transcriptional repression of nearby promoters. The requirement of *su(Hw)* for female fertility suggests that the protein normally regulates transcription during oogenesis, but targets (other than gypsy) have yet to be identified.

Null alleles of the posterior group gene *vasa* contain ovaries with degenerating egg chambers (Lasko and Ashburner 1988). *vasa* is a component of the polar granules localized at the posterior of the oocyte (see below) and is required for pole cell formation. Later, the protein becomes associated with the nuclear periphery, probably because it is a component of the germ-cell-specific "nuage" described in *Drosophila* and other organisms. Thus, *vasa* functions both in early nurse cell development and in pole cell formation. The protein encoded by *vasa* is a member of a family that includes several RNA helicases and translation factor EIF-4A. It may be complexed with RNA, either in polar granules or later when associated with the nuclear membrane of developing germ-line cells, consistent with a role regulating posttranscriptional steps in germ cell gene expression.

An interesting parallel can be drawn between early steps in oogenesis and preblastoderm embryonic development. In both cases, a defined number of divisions (four in the cyst and nine in the embryo) is followed by segregation of a germ-line lineage (the oocyte or the pole cells) from cells that will differentiate somatically (the nurse cells or the cellular blastoderm). Prior to segregation, cells destined for both germ-line and somatic fates are in cytoplasmic continuity. Cell fate in both systems may depend on cytoplasmically localized mRNAs and proteins, including Vasa. Subsequently, a series of cell-specific functions would take place that reinforce the choice and guide differentiation as an oocyte or nurse cell.

This general scheme provides potential explanations for many mutations that disrupt early oogenesis. If nurse cell versus oocyte cell fate is determined by localized determinants, then some early genes may encode these products, and others localize them. Failure to complete nurse cell development might also result from mutations in genes needed for nurse cell differentiation or from inappropriate expression of oocyte-specific functions in the nurse cells. For example, genes controlling polyploidization, DNA underrepresentation, and nucleolus formation must only be expressed in the nurse cells, whereas genes controlling meiotic arrest, karyosome formation, and endobody metabolism must be limited to the oocyte. Since only a handful of genes causing egg chamber degeneration have been analyzed, it is too early to deduce in molecular terms how oocyte versus nurse cell differentiation might be controlled. A possible clue is the regulation of *P*-element transposase production at the level of splicing; the third intron of the transposase gene can only be spliced in the germ line (Laski et al. 1986; Laski and Rubin 1989). Somatic-specific factors have been detected that appear to repress third intron splicing (Siebel and Rio 1990; for review, see Rio 1990).

E. Vitellogenesis

1. Yolk Production

Drosophila eggs accumulate large amounts of yolk during stages 8 through 10. Three major yolk constituents have been recognized: protein-containing particles, glycogen-rich particles, and lipid droplets. Protein-rich yolk granules

arise during a period when the oocyte membrane is accessible to the hemolymph due to a transient loosening of the follicle cell layer. Yolk precursor proteins (vitellogenins) are transported from their major site of synthesis in the fat body via the hemolymph and are subsequently taken up by endocytic vesicles at the oocyte plasma membrane (see DiMario and Mahowald 1986). In addition, a significant amount of yolk proteins are synthesized in the follicle cells themselves (Brennan et al. 1982). Vitellogenins appear to contain a signal that mediates their uptake by the oocyte and storage in stable vesicles (Yan and Postlethwait 1990). Lipid droplets also begin to appear during stage 8. Much of the neutral lipid stored in the egg is probably synthesized by nurse cells and transported into the oocyte, since the droplets first appear in nurse cells. In contrast, the glycogen-rich yolk particles do not appear until stage 13 and are probably synthesized in the oocyte in association with the mitochondria that surround them.

Three single-copy genes encode the major yolk proteins: YP1, YP2, and YP3 (Bownes 1990). YP1 and YP2 are encoded in region 9A by divergently oriented transcription units separated by a 1.2-kb region that controls expression in both the fat body and follicle cells. The YP3 gene also resides on the X chromosome (12B) and is regulated slightly differently than YP1 and YP2 (expression in follicle cells is reduced). All three proteins are posttranslationally modified following synthesis and entry into the secretory pathway via signal sequence cleavage (Warren et al. 1979; Minoo and Postlethwait 1985). The covalent addition of glycosyl, phosphate, and sulfate residues may likewise serve as a storage function. All three proteins are sulfated (Baeuerle and Huttner 1985); the single sulfation site on YP2 was mapped to Tyr-172 (Baeuerle et al. 1988). The functional significance of this modification is unknown, but a role in secretion has been suggested (Friederich et al. 1988).

The yolk supplies nutrients to the developing embryo. Vitellogenesis makes an extreme synthetic demand on adult females; under most circumstances, including common conditions in laboratory cultures, available food supply limits the number of eggs that can be produced. Yolk protein structure suggests that they contribute more than a source of amino acids, however. *Drosophila* vitellogenins show significant similarity between approximately residues 200 and 340 to several vertebrate proteins involved in lipid metabolism, including pig triacylglycerol lipase (Bownes et al. 1988) and human lipoprotein lipase (Baker 1988; Terpestra and Ab 1988). The region of similarity includes the lipid-binding region of the lipase active site. Although yolk proteins lack the active site, serine and lipase activity has not been detected, and enzymatic function for yolk proteins cannot be completely ruled out. A role in storing and releasing lipophilic molecules, particularly apolar conjugates of ecdysteroids to long-chain fatty acids, was favored, however. Release of yolk-protein-bound ecdysteroids may account for the embryonic peak of ecdysterone that is postulated to signal cuticle deposition. King (1970) recognized two types of protein yolk: a uniform particle and a particle that appeared to be filled with cylindrical crystalline subparticles. It is possible that the two represent particles contributed by the fat body and follicle cells, respectively, and possibly carrying different bound lipophilic molecules. Alternatively, the uniform particles may be a precursor to the crystalline ones (Mahowald and Kambsellis 1980). YP1 is also similar between amino acids 1–200 and *C. elegans* vitellogenin.

2. Regulation of Vitellogenesis

Synthesis of yolk proteins in the fat body is normally limited to females. Ovaries containing eggs ready to take up yolk are not required, since females bearing early mutations in oogenesis frequently contain high levels of vitellogenin in the hemolymph. Both ecdysone and juvenile hormone have been implicated in providing a hormonal signal (see Bownes 1989). Expression of YP1 in males using *hsp70* fusions demonstrated that YP1 transcripts are processed, and the protein efficiently secreted into the hemolymph (see Kraus et al. 1988). Thus, no evidence exists for sex-specific posttranslational regulation.

The yolk protein genes are downstream genes in the sex determination hierarchy. Several somatic sex determination genes are required to maintain as well as initiate yolk protein synthesis in the fat body (Bownes and Nöthiger 1981; Belote et al. 1985). These include the upstream gene *tra-2* (51B) and the most likely direct regulator, *doublesex* (84E) (Baker and Ridge 1980; Burtis and Baker 1989). Recently, both the male-specific and female-specific proteins encoded by *doublesex* were shown to bind to the fat body enhancer shared by YP1 and YP2 (Burtis et al. 1991). In contrast, *tra-2* (and probably other somatic sex determination genes) is not required to maintain yolk protein synthesis in the follicle cells, a female-specific tissue (Bownes et al. 1990). Transcription of yolk protein genes in the follicle cells is controlled by *cis*-regulatory sequences distinct from those regulating fat body expression (Garabedian et al. 1985).

Early genetic screens for female steriles frequently recovered mutations that appeared to disrupt vitellogenesis (see Mahowald and Kambyrellis 1980). Although many of these, including *apterous*⁴ (41B), could be partially corrected by exogenous juvenile hormone (Postlethwait and Handler 1978), these mutations now appear to act indirectly through deleterious effects on nonovarian tissues. More recently, specific mutations affecting vitellogenesis have been identified. Females homozygous for *fs(1) yolkless* (12E) accumulate large amounts of all three yolk proteins in the hemolymph, but very little yolk is taken up into the oocyte (Waring et al. 1983; DiMario and Mahowald 1987). This germ-line autonomous gene may encode a specific yolk receptor or it may encode some other component of a receptor-mediated endocytic pathway specific for yolk uptake.

Genetic tests have provided insights into the function of all three yolk genes. *fs(1)1163* is an EMS-induced recessive female sterile mutation affecting residue 92 of YP1 (Saunders and Bownes 1986) that becomes semidominant at elevated temperature (Bownes and Hodson 1980). The mutation alters YP1 secretion from the fat body (Butterworth et al. 1991), affects a YP1 posttranslational modification (Minoo and Postlethwait 1985), and leads to extensive abnormalities in follicle cells prior to vitelline membrane secretion (Giorgi and Postlethwait 1985), possibly due to the accumulation of the mutant YP1 protein. Mutations in the A59 complementation group (including K313) generate a similar phenotype by greatly reducing YP2 accumulation (S. Liddell et al., pers. comm.). However, another YP2 mutant, 12-1245, eliminates detectable YP2 in the ovary without abolishing fertility (Tamura et al. 1985). Furthermore, YP3 production is completely eliminated by the YP3⁵¹ mutation, but there is no effect on fertility (Liddell and Bownes 1991). These results are hard to reconcile with the hypothesis that YP1 and YP2 have unique functions, such

as transporting and storing different lipophilic molecules. It seems more likely that the sterility associated with some alleles results from disruption and damage to fat body or follicle cells caused by the mutant proteins. Expression of an YP-invertase fusion protein also caused dose-dependent, dominant sterility (Yan and Postlethwait 1990).

F. Follicle Cell Migrations

1. Migration over the Oocyte

At the start of stage 9, nearly all of the follicle cells move posteriorly in a concerted manner toward the oocyte. Only about 50 cells remain over the nurse cells. They flatten extensively so that the nurse cells remain completely covered by follicle cells. However, the covering is so thin that the follicle cell nuclei would bulge out were they not usually located within the small valleys created by the joining of two adjacent nurse cells. The nonmigrating cells can be seen to represent a separate population well before the onset of migration (see Fig. 6G). Following nurse cell breakdown, when main body follicle cells again move anteriorly, these lines continue to label a subpopulation of follicle cell nuclei that remain associated with the degenerating nurse cell nuclei. They probably correspond to the follicle cell nuclei observed in this region that did not synthesize chorion proteins (Parks and Spradling 1987). The mutant *tiny*, defined by a single allele, has been suggested to disrupt follicle cell migration over the oocyte (King 1970; see also DiMario and Hennen 1978). In cup or open chorion mutants, follicle cell migration also stops short of covering the oocyte (see Schüpbach and Wieschaus 1991). Subsequently, centripetal follicle cells do not migrate to form the anterior chorion, perhaps because they overlie nurse cells, rather than the nurse cell-oocyte border. These mutations could disrupt genes required for migration or simply slow the growth of the oocyte relative to the follicle cells, so that the migrating cells cannot all squeeze onto the oocyte surface.

To make room for the migrating cells, cells at the posterior end normally become more columnar in shape. Little is known about the mechanism responsible for the migration or the shape change. However, a simple model would postulate that a new high-affinity ligand appears on the oocyte at the end of stage 8 (or a new receptor on the follicle cell membranes). Because the migrating cells appear to remain interconnected at all times, they move only relative to their underlying substrates but not relative to their immediate neighbors. Thus, coordinated changes in the cytoskeleton within each follicle cell, in conjunction with an anchoring point at the posterior pole of the egg, might be sufficient to generate the migration. Follicle cells are the only adult cells that express $\beta 3$ tubulin, which in some embryonic and pupal tissues is associated with changes in cell shape (Kimble et al. 1989). Mutations in the $\beta 3$ gene (60C) have been recovered, but their lethality has prevented the function of this gene in follicle cells from being characterized (Kimble et al. 1990). *Drosophila* contains extracellular matrix proteins similar to those in vertebrates (for review, see Fessler and Fessler 1989), but their location in the egg chamber is poorly known and whether they function in cell migration has not been resolved. Mutations in the *myospheroid* gene eliminate production of the

Drosophila integrin β chain, are lethal, but do not disrupt oogenesis when present in germ-line clones (Leptin et al. 1989). However, it has not been possible to test for a possible role of integrins produced by somatic cells.

2. Border Cell Migration

A group of six to ten border cells undergoes a spectacular migration at the same time as the general movement toward the oocyte. These cells first become recognizable when they lag behind at the anterior end as the other follicle cells begin to move toward the oocyte. Shortly thereafter, the cells extend processes between two anterior nurse cells, move between the cells, and begin migrating toward the anterior tip of the oocyte. The border cells remain associated with each other as they migrate. During stage 9, the cells migrate nearly 150 μm (about 15 cell diameters). Some mechanism must guide their movement, since they travel directly toward the anterior end of the oocyte and pass at least three nurse cell junctions without making an incorrect choice. (Rarely a single border cell appears to become separated and wanders laterally between nurse cells.) After arrival, the border cells remain at the anterior end of the oocyte, although they move dorsally during stage 10B. Their role in producing the micropyle (see Fig. 6J) is described below.

Gene expression studies reveal that specialized groups of polar follicle cells are present at both ends of the egg chamber, beginning quite early in oogenesis (see Fig. 6). One of several antibodies originally prepared using imaginal disc membranes (Brower et al. 1980) heavily labeled just two follicle cells at each end of the egg chamber. This antigen is now known to be the product of the *fasciclin III* (36E) gene (Patel et al. 1987). Expression of *lacZ* in *fasciclin III* enhancer-trap lines appears in both the anterior and posterior cell pairs by stage 3 (see Fig. 6B). At least two other genes defined by enhancer-trap insertions also are expressed in polar cell pairs at each end of the follicle, whereas additional lines are expressed in larger sets of polar cells (D. Montell and A. Spradling, pers. comm.). The anterior members of these groups always include border cells, although the posterior members may share similar potential, since border cells migrate at both ends of dicephalic egg chambers (Lohsschardin 1982). Expression of *torsolike* (93F) in polar follicle cells is likely to activate the *torso* receptor at the embryonic termini (see Casanova and Struhl 1989; Sprenger et al. 1989; Stevens et al. 1990). An enhancer-trap insertion in *torsolike* is expressed in border cells and in a larger subset of posterior polar cells (D. Montell and A. Spradling, pers. comm.). The *fasciclin-III*-positive cells have been postulated to play a role in determining the fate of surrounding cells (Ruhola et al. 1991).

The specificity of the border cell migration makes it an attractive model system for studying how cells move through heterologous cells to a specific destination. Do border cells follow signals on nurse cell surfaces or sense an attractant from the oocyte? In small egg mutants, where follicle cells do not finish migrating over the abnormally small oocyte, the border cells also stop short, suggesting that their migration remains coordinated with the movement of follicle cells (Schüpbach and Wieschaus 1991). Border cell movement is not inhibited by colchicine (Koch and Sptizer 1983). Insertion mutations eliminating *fasciclin III* expression in border cells do not affect their migration and are fertile (D. Montell and A. Spradling, pers. comm.).

Border cells form but fail to migrate in egg chambers from *slow-border-cells*

(*slowo*) (60C) homozygotes (Montell et al. 1992). In the presence of weak alleles, migration is delayed until stage 10 or later, suggesting that a threshold level of *slowo* product is required to initiate migration. The gene encodes a homolog of the mammalian C/EBP transcription factor. During oogenesis, both RNA and protein are specifically expressed in border cells, beginning several hours prior to the onset of migration, and are no longer found after stage 10. *slowo* expression may control genes whose products actually power and guide border cell movement. The gene is also active in a subset of centripetally migrating follicle cells; however, their movement over the anterior surface is not disrupted in mutants.

G. Nurse Cell-Oocyte Transport

Nurse cells provide the oocyte with the vast majority of its cytoplasmic constituents. Vast numbers of ribosomes and mitochondria are synthesized in nurse cells and transferred to the oocyte. Because electron microscopy sections of germania revealed large organelles such as mitochondria within the ring canals joining cystocytes, a general flow of materials termed the nutrient stream has generally been assumed to move continuously and unselectively from nurse cells toward the oocyte throughout oogenesis. Just prior to nurse cell breakdown, a massive stream can indeed be seen entering the oocyte, where its force briefly excludes yolk spheres from the adjoining ooplasm. However, more recent studies that followed the fate of specific RNAs have revealed that egg chambers do control the movement of materials between the nurse cells and oocyte.

Two distinct phases of transport were suggested by studies of histone mRNAs within the egg chamber (Ruddell and Jacobs-Lorena 1985; Ambrosio and Schedl 1985). Prior to stage 10, histone mRNAs appear to undergo periodic synthesis and breakdown in nurse cells, but only very low levels can be detected in the oocyte. During this time, a discontinuity in histone mRNA concentration (frequently) exists across the ring canals separating the nurse cell and oocyte cytoplasm, suggesting that these mRNAs are not flowing freely in a nutrient stream. Subsequently, during stage 10, histone mRNAs accumulate to high levels and are transferred to the oocyte with the rest of the nurse cell contents at breakdown. A variety of other mRNAs, including cytoplasmic actin mRNA and *otu* mRNA, have been observed to follow a similar pattern. Some genes, such as *bam*, only begin to produce RNA in nurse cells at stage 10B for storage in the oocyte. These observations suggest that synthesis during the early phase may be primarily for use within the nurse cells, whereas synthesis at stage 10B may be primarily for storage in the egg. Of course, some early RNAs are likely to be transferred to the oocyte to support its steady growth in size. However, each nurse cell would have to contribute only 7% of its output to maintain the size of the oocyte. Furthermore, it is not known if the early oocyte has a normal complement of cytoplasmic constituents, and some oocyte growth after stage 7 is likely to be due to yolk uptake.

Certain gene products show a very different pattern of accumulation during oogenesis. Transcripts of the genes *bicoid*, *Bic-D*, *K10* (2E), *oskar*, *orb*, *BJ1*, and *hts* all accumulate precociously in the oocyte as well as in nurse cells. In

early egg chambers, the situation is complicated by the possibility that the oocyte nucleus contributes to synthesis. However, between stages 3 and 10, *bcd*, *oskar*, and *Bic-D* are transcribed in nurse cells, but much of the RNA appears to move rapidly into the oocyte through the ring canals. These studies suggest that a second class of RNAs may be transported preferentially through ring canals, during the same time that other transcripts are excluded. Strong evidence supporting this conclusion has been reported in the case of *K10* transcripts (Cheung et al. 1992). Several of these RNAs eventually become localized in the oocyte (see below). *oskar* RNA, which is localized at the posterior of mature egg, transiently accumulates at the anterior of the oocyte in stage-8–10 egg chambers. *orb* RNA, which is not localized in mature eggs, also temporarily accumulates in a similar manner. A pool of these RNAs may build up in early vitellogenic oocytes, because the transport mechanism bringing them in from the nurse cells temporarily exceeds the capacity of the oocytes' mechanism to transport them posteriorward.

1. Mechanism of Early Transport

Several mechanisms have been proposed to power material through the ring canals into the oocyte as the egg chamber is growing prior to nurse cell breakdown. The morphology of the ring canals provides few clues as to this mechanism. The ring canals enlarge from less than 1 μm to greater than 10 μm in diameter during egg chamber growth, and they accumulate large amounts of filamentous actin that allow them to be visualized conveniently using rhodamine-conjugated phalloidin (Fig. 8) (Warn et al. 1985). Material from distant nurse cells must pass through as many as three separate ring canals before entering the oocyte (see Fig. 5); possibly for this reason, a size gradient exists, with the largest canals joining the oocyte. The four oocyte ring canals are drawn together just prior to nurse cell breakdown. The functional significance of these changes remains uncertain, however.

The idea that electrical potential differences power transport has received considerable attention. Voltage gradients between nurse cells and the oocyte were first reported and proposed to explain transport in the moth *H. cecropia* (Woodruff and Telfer 1980). Similar potential differences were reported to exist in *Drosophila* egg chambers (Overall and Jaffe 1985). However, other studies (Bohrmann et al. 1986b,c; Sun and Wyman 1987, 1992) found very little potential differences between *Drosophila* nurse cells and the oocyte and documented variability induced by the experimental conditions used during measurement. Fluorescently labeled acidic and basic proteins microinjected into nurse cells or oocyte of vitellogenic follicles did not accumulate in a charge-dependent manner (Bohrmann and Gutzeit 1987). Thus, available evidence does not support a role for electrophoresis in the early phases of transport.

Recent evidence supports a role for microtubules in transporting materials from the nurse cells into the oocyte. Microtubules have been visualized within ring canals. Oocyte growth (Koch and Spitzer 1983) and localization of mRNAs within the oocyte are disrupted by inhibitors such as colchicine and taxol that disturb microtubule function (Pokryoka and Stephenson 1991). Mitochondria, which have frequently been seen passing through ring canals in electron micrographs, are known to be transported along microtubules through the action of kinesins. The class of RNAs that enter the oocyte early may be transported by kinesin-like molecules to which they bind. A micro-

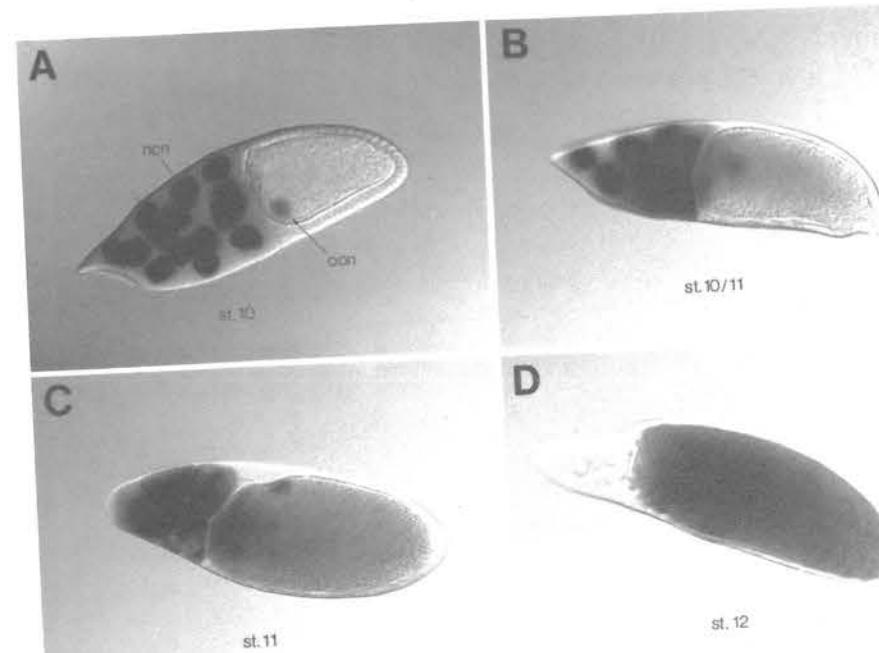


Figure 8 Nurse cell breakdown. X-gal staining of an enhancer-trap line with strong germ-line expression is illustrated at four successive stages before, during, and after nurse cell breakdown. (A) Stage 10B; (B) stage 10B/11; (C) stage 11; (D) stage 12. Magnification, ~150 \times . (Photos courtesy of L. Cooley.)

tubule-based mechanism would be a natural extension of the system thought to transport materials differentially in early germarial cysts during the process of oocyte determination. The transient buildup of mRNAs at the oocyte anterior might result from temporary saturation of the microtubules leading toward the posterior.

2. Nurse Cell Breakdown

The process of nurse cell breakdown rapidly moves massive amounts of nurse cell products into the oocyte, nearly doubling its volume in less than 30 minutes (see Fig. 8). Considerable evidence now suggests that the existing transport mechanism is modified or perhaps even replaced to accomplish this. In stage-10B chambers (Fig. 9), a system of actin-based filaments forms within the nurse cells (for review, see Gutzeit 1986a). Microfilament bundles up to 30 μm in length are observed that connect the nurse cell nuclei and plasma membranes (Fig. 9B). Cytoplasmic oscillations and streaming increase at this time in both the nurse cells and oocyte (Gutzeit and Koppa 1982). Nurse cell dumping is prevented by treatment with cytochalasin B, a drug that also prevents microfilament formation and nurse cell cytoplasmic oscillations (Gutzeit 1986b). In contrast, treatment with colchicine, a microtubule inhibitor, abolishes cytoplasmic streaming in the oocyte but does not prevent nurse cell breakdown (Gutzeit 1986a). Following nurse cell breakdown, the remnants of this system, including fibrous masses of actin, remain outside the oocyte,

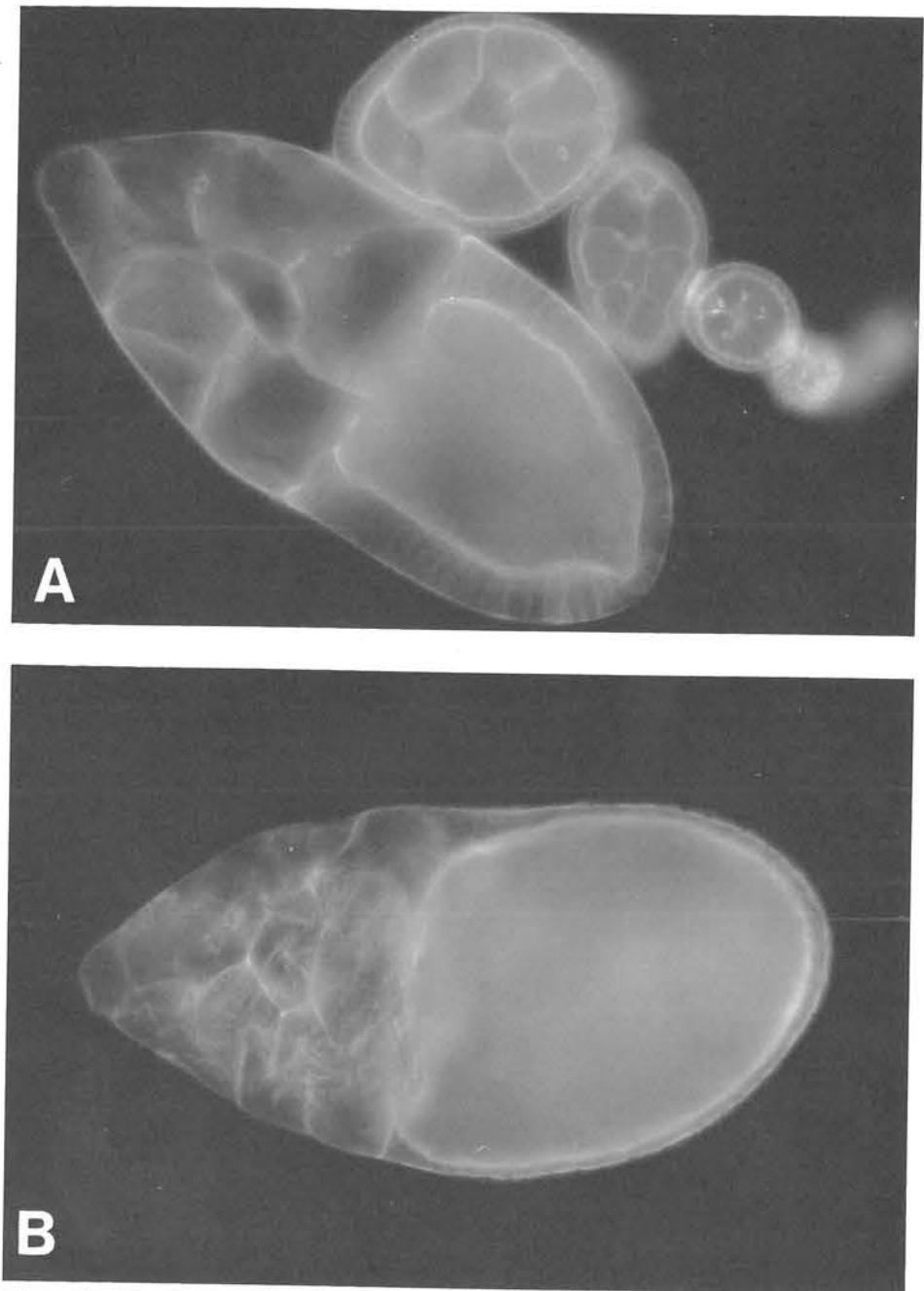


Figure 9 Egg chambers stained with rhodamine-labeled phalloidin (see Warn et al. 1985) to reveal actin-rich structures. (A) Egg chambers with ring canals; (B) stage II chamber with actin filaments in degenerating nurse cells. Magnification, ~300x. (Photos courtesy of L. Cooley.)

where they gradually disappear among the degenerating nurse cell nuclei. One interpretation of these observations is that contraction of the actin filaments drives the nurse cell cytoplasm through ring canals and into the oocyte (Gutzeit 1986b). However, careful measurements of filament lengths have not

detected evidence of contraction at the time breakdown begins (W. Theurkauf, pers. comm.).

Several genes have been identified that may play important roles in nurse cell breakdown. Females homozygous for *singed*, *chickadee*, *kelch* (36E), or *quail* (36C) produce egg chambers that do not dump most of the nurse cell contents into the oocyte during stage 11 ("dumpless" mutations). Multiple alleles of all of these genes display similar defects; however, it is not yet known if these represent null phenotypes. Development continues in mutant chambers, so that an eggshell is synthesized around smaller-sized eggs. The dorsal appendages are frequently abnormal, probably because remnant nurse cell cytoplasm interferes with the migrating follicle cells.

Cloning of *singed* (Paterson and O'Hare 1991) revealed that the locus expresses RNAs encoding a 57-kD protein in stage-9 and -10 nurse cells (and in a few follicle cells). Since the locus is germ-line-dependent, the expression in nurse cells is likely to be responsible for the failure to transfer nurse cell cytoplasm. The appearance of *singed* mRNA at stage 9 suggests that it may be involved in assembling the actin filament system. The involvement of *singed* in bristle morphogenesis is consistent with this proposed function, since bristle elongation also involves abundant microfilaments. Recently, both the *chk* and *kel* genes have been cloned as well (Yue and Cooley 1990; Cooley et al. 1992). *chk* encodes the *Drosophila* profilin gene, a highly conserved protein whose interactions with actin and involvement in IP₃-mediated signal transduction pathways have been extensively studied (see Majerus et al. 1990).

More detailed study of dumpless egg chambers have supported an alternative view for the function of the nurse cell actin filaments. These filaments never form in homozygous *chk* egg chambers. In these egg chambers, nurse cell nuclei move toward the oocyte and become lodged within the oocyte's ring canals, which they "plug up" (Cooley et al. 1992). The actin filaments may therefore serve to tether the nuclei away from the ring canals, since they are too large to pass through. This could explain why nurse cell nuclei are excluded from the oocyte and complete their degeneration between the developing dorsal appendages. This model does not require that the filaments undergo contraction.

What then drives the breakdown process? A reasonable hypothesis would be the force that drives egg chamber elongation. Nurse cell breakdown is accompanied by rapid flattening of the columnar follicle cells that overlie the oocyte, causing the egg chamber to increase substantially in length between stage 10B and stage 12. Since the nurse cell nuclei and membranes are tied together by the actin filaments, they may be simultaneously driven anteriorly. This would force cytoplasm through the ring canals and enlarge the oocyte. Unlike transport from nurse cell to oocyte, this model suggests that the anterior oocyte and nurse cell membranes are drawn through a fixed mass of cytoplasm, much as one could push a piston into a water-filled cylinder if the piston had a few holes in its surface. Both microtubules and microfilaments in the follicle cells have been suggested to participate in egg chamber elongation; the issue remains unresolved (Gutzeit 1990).

H. Eggshell Formation

The *Drosophila* eggshell resembles that of many insects whose embryos develop externally (Hinton 1981). The shell must be strong enough to support the egg in a hostile environment. Water loss must be minimized despite providing

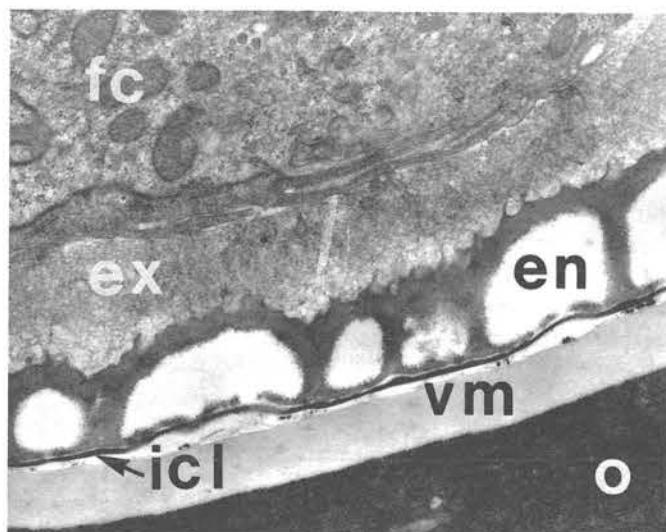


Figure 10 Electron micrograph of a stage-14 egg chamber section showing the structure of the eggshell. The oocyte (o), vitelline membrane (vm), inner chorionic layer (icl), endochorion (en), exochorion (ex), and overlying follicle cell (fc) are indicated. Magnification, 150,000x. (Photo courtesy of Drs. G. Waring and A. Mahowald.)

for gas exchanges essential to embryonic respiration. Insects provide these functions to their eggs by coating them with several specialized shell layers (Fig. 10). From the oocyte moving outward, these consist of the vitelline membrane, the wax layer, the inner chorionic layer (ICL), the endochorion, and the exochorion. The vitelline membrane in a mature egg is a rigid structure quite capable of maintaining the shape of the egg even after removal of the outer-layers. The endochorion forms the major structural and gas-transporting component. Air passageways encircling the egg are maintained in this layer by a series of pillars that keep the thin porous "floor" of the endochorion separated from its substantial roof. The endochorionic air channels open to the outside through areopyles; in *Drosophila*, a few small areopyles are located in a region of specialized chorion at the posterior pole and in two major structures: the dorsal respiratory horns and dorsal appendages. The thin ICL, which has a crystalline structure (Akey and Edelstein 1987; Akey et al. 1987), and the wax layer appear to function in waterproofing the egg just prior to laying. Additional structural strength results from extensive posttranslational modifications and cross-linking of the shell proteins. Prior to stage 14, chorion proteins can be solubilized by detergent; this is not true for eggs that have passed through the oviduct. The chorion and vitelline membrane remain largely separate since the chorion can be readily removed, even in mature laid eggs. For further information on eggshell structure, see Margaritis (1985a).

The follicle cells do a remarkably precise job of building the eggshell during the final day of oogenesis (stages 8–14). The micropyle, for example, is a precise length and taper and contains a central pore channel that passes through the micropyle cone at an eccentric angle and terminates near a "vitelline protrusion" that forms a specific "pocket" within the vitelline cone. How such precise structural features are constructed remains a fascinating problem. The

eggshell certainly does not self-assemble. Rather than relying on intrinsic properties of the component proteins, the shell is built by correctly positioning subgroups of follicle cells that are responsible for individual portions of the structure. For example, the endochorionic pillars are postulated to correspond to the position of follicle cell surface protrusions where chorion proteins are secreted. Specialized cells that migrate, the border cells and certain centripetally migrating cells, cooperate to form the micropyle. Much larger groups of cells lay down the dorsal appendages progressively, from base to tip, as they migrate anteriorly. Thus, follicle cells build the shell by precisely migrating, changing their shape, tailoring their cell surface properties, and integrating structural gene expression with these activities. Such changes can hardly be autonomous, but they require extensive information transfer between individual follicle cells and between the follicular layer and the underlying oocyte and nurse cells. Eggshell production therefore provides an extraordinary model system for the integration of many aspects of cell and developmental biology.

1. Vitelline Membrane Synthesis

Eggs can develop quite well surrounded only by the vitelline membrane and its wax layer coating. However, when egg permeability is increased by treatment with hydrocarbons that remove the wax layer, development can only proceed under rigorously maintained conditions of optimum relative humidity. The vitelline membrane reflects the polarity of the developing egg chamber. Its dorsal surface is flattened, and the anterior and posterior ends are distinctively rounded. The vitelline portion of the micropylar cone lies at the anterior end. Recently, it has been suggested that positional signals for embryonic pattern formation may be embedded during oogenesis in the vitelline membrane, the portion of the shell directly adjacent to the oocyte plasma membrane (Stein et al. 1991). Such signals would be permanently stored but accessible to the egg for use during embryogenesis (see Chasan and Anderson, this volume).

The protein components of the vitelline membrane have been analyzed, and their temporal regulation has been studied. All known vitelline membrane constituents are synthesized by the follicle cells. They begin to be secreted during stage 9A and accumulate in small vesicles called vitelline bodies that lie between the oocyte and the overlying follicle cells. The vitelline bodies remain separate and therefore do not interfere with yolk uptake from the hemolymph by the oocyte plasma membrane. During stage 10B, vitelline body fusion is rapidly activated. The vesicles coalesce to form a rather uniform layer nearly 2- μ m thick. The timing of secretion and fusion of vitelline bodies is not spatially uniform. The vitelline membrane forms more slowly at the anterior end, and its thickness is modulated, both near the posterior areopyles and underlying the micropyle, although region-specific proteins have not been demonstrated. A *Vm32E-lacZ* fusion gene was preferentially expressed in main body cells compared to terminal follicle cells (G. Gargiulo et al., pers. comm.), providing additional evidence for spatial regulation of vitelline membrane structure.

Vitelline membrane is composed of a small number of abundant proteins (*Sv14*, *Sv17.5*, and *Sv23*) and an undetermined number of minor constituents (Fargnoli and Waring 1982; Burke et al. 1987). Both *Sv17.5* and *Sv23*, as well as

two putative minor vitelline membrane proteins, are encoded in a gene cluster at 26A that spans at least 8 kb. Single genes encoding two other vitelline membrane proteins are at other sites (Mindrinos et al. 1985; Gigliotti et al. 1989). All of the characterized abundant vitelline membrane proteins are rich in proline and alanine and contain a similar hydrophobic domain (Scherer et al. 1988). This region is so highly conserved that cross-hybridization between the genes is readily detected. Genes encoding these proteins all lack introns. Despite these similarities, at least some proteins probably carry out distinct functions. The temporal expression profiles within the stage-8 to stage-11 window for vitelline membrane production differ between some individual genes. Furthermore, the *fs(2)QJ42* mutation, which is rescued by a 6.5-kb fragment containing the *Sv23* gene (Savant and Waring 1989), specifically eliminates the accumulation of *Sv23* mRNA and protein and causes abnormal vitelline membranes that collapse when the chorion is removed. Even a reduced amount of *Sv23* produced by a single copy of a transposon containing only 147 bp 5' to the gene rescued the phenotype. Thus, the collapsed egg phenotype does not appear to result simply from a general decrease in vitelline membrane proteins but suggests that *Sv23* plays a specific role in vitelline membrane function.

Many lethal mutants produce collapsed eggs in germ-line clones. Some of these may participate in vitelline membrane assembly. *fs(1)Nasrat* and *fs(1)polehole* (5C) homozygotes frequently produce collapsed eggs, and fertilized eggs show defects in the terminal pathway (Degelman et al. 1986; 1990). Both phenotypes are autonomous to the germ line, so the genes cannot simply encode vitelline membrane proteins secreted by the terminal follicle cells. However, the *Nasrat* and *polehole* products may be assembled into the oocyte plasma membrane or transported into the perivitelline space where they stabilize the vitelline membrane. Many other lethals that produce collapsed eggs in germ-line clones appear to do so nonspecifically. Perhaps if the metabolic health of the oocyte is compromised, it cannot take up water and expand fully during egg maturation, a process that may be required to render the vitelline membrane impermeable.

2. Chorion Production

The chorion portion of the eggshell actually comprises the channels for gas exchange, including the dorsal appendages. Studies of the molecular biology (for review, see Osheim 1985; Kafatos et al. 1987; Spradling and Orr-Weaver 1987; Orr-Weaver 1991) and morphology (for review, see Margaritis 1985a) of chorion production have helped elucidate mechanisms of chorion morphogenesis. Of particular importance has been an improved understanding of chorion protein structure. Six major structural proteins encoded by single-copy genes exhibit comparable acid composition but little sequence similarity. Two major and four minor chorion genes are clustered on the X chromosome (7F) (Parks et al. 1986), whereas the four other major proteins are encoded in a second cluster on chromosome 3 (66D). A report of an unlinked chorion gene (Yannoni and Petri 1984) was erroneous, but additional minor structural proteins do undoubtedly exist. On the basis of their times of expression (Parks and Spradling 1987), the X-linked genes are thought to encode ICL and endochorion components, whereas the proteins encoded by the third chromosome genes probably function in the exochorion.

Evolutionary comparisons have revealed potentially important protein domains. The four genes in the third chromosome cluster have been sequenced in four species of *Drosophila* (Martinez-Cruzado et al. 1988; Fenerjian et al. 1989); the X cluster proteins, s36 and s38, have been compared in *Drosophila virilis* and the distantly related medfly *Ceratitis capitata* (Konsolaki et al. 1990; Tolias et al. 1990). Overall, there was strong conservation of gene organization, so that homologs of the genes in the two clusters could be identified. Within the third chromosome cluster, there was relatively little conservation at the protein sequence level. However, the s36 and s38 proteins were strongly conserved in a central domain (95% identity within amino acids 97–182 between *Drosophila* and medfly s38 (Tolias et al. 1990). The greater conservation of X-linked than chromosome-3 chorion genes is in accord with several facts. Mutants have been recovered in s36 (Digan et al. 1979) but not in any members of the third chromosome cluster. The endochorion also appears much more similar between diverse insects than the exochorion. s36 may form endochorion pillars since these are lacking in *cor36* mutants, which abolish production of this protein. Analysis of *In(1)ocelliless/fs(1)cor36* eggs revealed that a relatively small amount of s36 protein, 10–20% of wild type, is sufficient for fertility (Digan et al. 1979; Parks et al. 1986). s38 appears to be the main eggshell peroxidase and may be located primarily in the ICL (Keramaris et al. 1991).

3. Cross-linking and Morphogenesis

The eggshell forms intricate structures with a high degree of accuracy, providing a model system for studying the molecular mechanisms controlling morphogenesis in eukaryotes (Regier and Kafatos 1985). Eggshells are shaped by the action of specific follicle cells that separate into subgroups, move to specific positions, modify their surfaces, and secrete a series of products under tight temporal control. Morphogenesis continues extracellularly as eggshell proteins are cleaved and cross-linked. The eggshell remains pliable and slightly folded in the ovary. The egg does not assume its final shape until it takes up additional water during passage in the oviduct.

The eggshell is stabilized by a progressive cross-linking process that renders its proteins largely insoluble by the end of stage 14. Peroxidase-catalyzed di- and tri-tyrosyl cross-links strengthen the eggshell (Petri et al. 1979); egg chambers that are allowed to develop *in vitro* in the presence of phloroglucinol, a peroxidase inhibitor, never become insoluble (Mindrinos et al. 1980). Cross-links of this type are common in the eggshells of insects and other organisms. Proteins with peroxidase activity, which may represent primarily the s38 protein, appear in the eggshell beginning late in stage 10, where they may also play a structural role (Giorgi and Deri 1976; Keramaris et al. 1991). Cross-linking is thought to initiate later due to hydrogen peroxide production within the follicle cells (Margaritis 1985b). Cross-linking begins first at the termini, including the dorsal appendages.

The *dec-1* (7C) locus encodes proteins of special importance for chorion morphogenesis (Komitopoulou et al. 1983; Lineruth and Lambertsson 1986b; Bauer and Waring 1987; Waring et al. 1990). Mutants that remove all *dec-1* function are morphologically normal until stages 13 and 14, when the chorion fragments and intrudes into the previously normal vitelline membrane. At least three *dec-1* mRNAs encoding related proteins are produced by differ-

tial splicing. Additional proteins result from proteolytic cleavage. Both splicing and cleavage are temporally regulated. A major product, fc106, is produced while the vitelline membrane is still being laid down. It is rapidly cleaved to yield a vitelline-membrane-like 29-kD protein of unknown function and a major chorion structural protein s80. s80 probably undergoes still further cleavages as the chorion is assembled in stages 13 and 14. In addition, *dec-1* encodes larger proteins of predicted size, 177 kD and 125 kD; at least one of these proteins or their cleavage products is important for eggshell assembly, since the *Df(1)ct^{4b1}* allele that produces only fc106 does not support fertility. *fs(1)1501*, a splice acceptor mutant that produces only the larger proteins, complements *Df(1)ct^{4b1}*. However, *fs(1)1501* homozygotes are sterile despite morphologically normal eggshells, indicating that fc106 derivatives play a role in morphogenesis distinct from the larger proteins.

Waring et al. (1990) proposed that the *dec-1* products are involved in additional cross-linking of the vitelline membrane and chorion. fc177 contains a unique cysteine-rich carboxy-terminal domain that may become linked to the cysteine-rich vitelline membrane proteins. In contrast, the high glutamine content of the fc106-derived s80 protein is reminiscent of involucrin and similar proteins that stabilize protein networks by transglutaminase-catalyzed linkages between glutamines and primary amines (e.g., lysines). The s36 and s38 proteins are excellent candidates for such cross-linking, since they contain at least eight lysines within their highly conserved central domains. (Interestingly, the likely tyrosine substrates of the peroxidase-catalyzed cross-links lie mostly outside this domain.) s80 cleavages are proposed to drive sequential cross-linking steps. Thus, the *dec-1* locus may integrate and stabilize the final assembly of both vitelline membrane and chorion layers.

4. Micropyle Formation

The formation of specialized eggshell regions such as the micropyle requires even more cellular sophistication than the main eggshell layers. A group of 40–50 follicle cells that derive from two distinct subpopulations produce the micropyle (Margaritis 1985a; Zarani and Margaritis 1986). This cone-shaped sperm conduit is produced to highly reproducible and exacting standards; both the lower vitelline portion and overlying endochorionic cone are pierced by a pore canal at an eccentric angle that provides passage into the egg and also contains a cul-de-sac ("the vitelline protrusion") of unknown function. The micropyle works so well that a sperm has never been visualized in transit.

Zarani and Margaritis (1986) have reconstructed the cellular events of micropyle formation. The process begins when six to ten border cells complete their migration through the nurse cells and reach the anterior end of the stage-10A oocyte. They secrete a blob of "paracrystalline" material before backing off slightly as 30–40 centripetally migrating follicle cells arrive. One or two border cells extend a process of microtubule-filled cytoplasm, whose tip remains associated with the paracrystalline material as the border cells retreat just anterior to the centripetal cells. The pore channel is formed and shaped by these border cell processes, as vitelline membrane and chorion are secreted around them by both sets of cells.

Recently, it has been possible to verify and extend our understanding of micropyle formation through genetic and laser ablation experiments (Montell et al. 1992). Surprisingly, when border cells are prevented from reaching the

anterior of the oocyte by ablation, or when their migration is blocked by mutation at the *slobo* locus, a micropyle still forms at the correct position. Thus, border cells are not required to initiate micropyle production. However, micropyles produced without border cells are defective; they completely lack a pore channel, accounting for the sterility of *slobo* homozygotes. In addition, the paracrystalline material is absent from the vitelline cone and the endochorion is reduced and somewhat rounded, as expected if the border cells do contribute some materials to the final structure. A specific antigen located in border cells and at the tip of the micropyle (White et al. 1984) probably represents one such product that might function as a sperm receptor. Ectopic micropyles are induced at the posterior end of many dicephalic egg chambers and also in strong alleles of ventralizing genes such as *gurken*. At least in the latter case, ectopic "border cells" do not participate; the resulting micropyles lack a pore canal as in *slobo* homozygotes (A. Spradling, unpubl.).

5. Dorsal Appendage Formation

The dorsal filaments, which are elaborate specializations of the chorion, are part of the anterior eggshell structures that also include the operculum, its specially weakened collar designed to open on three sides before an exiting larva, and the micropyle. Each dorsal filament is formed by a population of approximately 150 follicle cells that start out over the anterior part of the oocyte at stage 10B (see Fig. 6). Filament follicle cells include many of the stage-10B follicle cells whose increased length allows the dorsal side of egg chambers to be recognized (see Fig. 6H). These cells begin to secrete the filament bases, the regions where they will attach to the main body of the eggshell before they begin migrating anteriorly (see Fig. 6I). Apparently, as cells migrate past the growing end, they join a cylinder of cells secreting chorion proteins and commence secretion themselves (Fig. 6E) (King 1970). Chorion protein synthesis must therefore be controlled in an anterior-posterior gradient along the developing filament; cells closer to the base would carry out later steps than more recently arrived distal cells. These movements are coordinated with and probably depend on the dramatic elongation of the egg chamber that occurs during stages 11–13. Certainly, full-length filaments are never seen on short eggs. Dorsal filament formation has also been reported to be sensitive to colchicine, indicating a potential role for microtubules (Koch and Spritzer 1983). The distal third of each appendage contains flattened regions ("paddles") that function as a gill (plastron) to support gas exchange in eggs deposited under water. The plastron is achieved by breaking the endochorionic roof into closely spaced plates, each containing supporting pillars that connect through a meshwork to other plate-bearing pillars. These regions only become distinctly different in appearance after the egg passes through the oviduct, suggesting that maturation and cross-linking events may be involved.

Formation of the dorsal appendages and other anterior chorionic structures is disrupted by mutations that disturb the dorsal-ventral pattern of both the eggshell and the oocyte (for review, see Manseau and Schüpbach 1989a). Filament formation behaves like a specific dorsal-ventral cell fate programmed along the dorsal-ventral axis of the follicle cells. Dorsalized chambers such as those produced by *K10*, *squid* (87F), *capu* (24D), and *spire* mutations contain an increased amount of dorsal appendage material and a reduced number of

main body follicle cells (Manseau and Schüpbach 1989b). Presumably, an increased number of cells are signaled to migrate and participate in appendage synthesis based on changes in dorsal-ventral signals, leaving fewer over the main portion of the eggshell. The ventralized egg chambers produced by *gurken* and *torpedo* homozygotes have more main-body follicle cells but produce no or very reduced dorsal appendages.

The positioning of dorsal filaments is also fixed along the anterior-posterior axis, since they normally form only at the anterior end. Signals from the germinal vesicle, which plays a key early role in dorsal-ventral patterning (see Mansseau and Schüpbach 1989a; Montell et al. 1991), also appear to specify the formation of filaments and associated cuticular structures (such as the collar) along the anterior-posterior axis. For example, although dicephalic egg chambers produce micropyles at both ends, dorsal filaments only arise at the end containing the germinal vesicle (see Frey and Gutzeit 1986). In eggs producing cup-shaped chorions, rudimentary dorsal filaments often form in the posterior half of the egg (Schüpbach and Wieschaus 1991). The spectrum of defects in these eggs is likely to result from retarded oocyte growth (A. Spradling, unpubl.). The undersized oocyte occupies only the posterior part of this eggshell, so the germinal vesicle can induce filaments only in this region. Abnormal germinal vesicle positioning may also explain the posterior location of dorsal appendages in certain alleles of ventralizing genes such as *gurken* (A. Spradling, unpubl.). For reasons that are not understood, posterior filaments are greatly reduced in size and confined to the dorsal midline.

Another substantial class of sterile mutations affects dorsal appendages without changing the pattern of the entire eggshell (*ne1* [1-2.5], *rhino*). The great majority of these mutations are unlikely to disrupt production of appendage-specific structural proteins, since such proteins have not been biochemically detected (Mindrinos et al. 1980; Waring et al. 1983). Filament formation may be a sensitive process, whose completion can be disrupted by nonspecific mechanisms (Komitopoulou et al. 1988). Indeed, many mutations that produce collapsed eggs also affect filament morphogenesis. Filament formation is also frequently abnormal during egg chamber development in vitro (Petri et al. 1979). This type of sensitivity appears to be rather common in development; for example, eye development is disrupted by many semilethal mutations, yielding rough-eyed adults. A large fraction of these "nonspecific" filament mutations are germ-line-dependent (see Galanopoulos et al. 1989). Filament development may depend on mechanical properties of the oocyte that are easily altered. Enough "specific" mutations exist that differ from the common filament-defective phenotype to allow this pathway to be analyzed genetically. For example, the somatic-dependent mutation *fs(1)del2* (2B) has reduced chorionic appendages (Orr et al. 1989). It is an allele of the *Broad* complex, genes involved in the early ecdysone response (see Shea et al. 1990). Changing the dosage of the X-linked chorion gene cluster reproducibly alters average filament length (Digan et al. 1979). Amplification mutations that generally reduce chorion protein production also decrease filament length.

6. Chorion Gene Amplification

The unusual storage requirements of eggs sometimes cannot be met by the dosages of genes characteristic of the germ line. In many species, specific

genes such as rDNA are amplified to augment the production of stored components. In *Drosophila*, neither the 5S nor rDNA genes are amplified during oogenesis (Jacobs-Lorena 1980). However, both the X and third chromosome chorion gene clusters do specifically amplify in follicle cells beginning more than 10 hours before the onset of gene transcription (for review, see Spradling and Orr-Weaver 1987; Kafatos et al. 1987; Orr-Weaver 1991). Increased gene copy number is required to produce chorion mRNAs at the rates required to occupy virtually all of the follicle cell ribosomes during a mere 1-2-hour synthetic period. In contrast, vitelline membrane genes are active for about 15 hours, the *dec-1* gene for about 10 hours, and YP genes for more than 15 hours. None of these genes are amplified. Similar considerations undoubtedly explain why chorion genes are amplified in a similar manner in all *Drosophila* species tested (Fenerjian et al. 1989) and even in some distant Dipterans such as the medfly (Tolias et al. 1990). The chorion genes in several moth species (for review, see Kafatos et al. 1987) are not amplified. These genes are transcribed over much longer time periods, are part of large families of genes that may be functionally redundant, and probably do not correspond directly to the *Drosophila* chorion genes, since they contribute to an exaggerated outer eggshell layer not seen in *Drosophila*.

The developmental specificity and large magnitude of amplification have made it a model system for studying DNA replication in a multicellular eukaryote. Replication forks initiate at multiple sites within the third chromosome gene cluster rather than at a single specific origin (Delidakis and Kafatos 1989; Heck and Spradling 1990). Several genes (*mus101* [12B], *fs(3)272* [3-47], *fs(3)293* [88B], and *chiffon*) are required during oogenesis to facilitate amplification (Orr et al. 1984; Kelley and Spradling 1986; Snyder et al. 1986; Tower and Spradling 1990). Amplification mutants produce a characteristic thin chorion syndrome that becomes more extreme as amplification is reduced, although a low level of fertility usually remains until amplification falls nearly to zero. *mus101* and *fs(3)293* also mutate to lethality and appear to encode functions required in the diploid cell cycle, since mutant disc cells display defective chromosome condensation (Gatti et al. 1983) or fragmented chromosomes (Szabad and Bryant 1982). For a detailed discussion of the regulation of amplification, see Orr-Weaver (1991).

7. Transcriptional Control

The sequential expression of a long series of abundant structural proteins by the follicle cells provides a highly favorable system for studying the genetic regulation of gene transcription. DNA sequences responsible for sex-specific fat body synthesis and for follicle cell expression have been mapped upstream of YP1 and YP2 (Garabedian et al. 1985; Logan et al. 1989; Logan and Wensink 1990). The paired genes are controlled by common regulatory elements. A 125-bp enhancer located upstream of YP1 acts bidirectionally to specify fat body expression. A more complex *cis*-regulatory region surrounding the YP2 transcription start site controls follicle cell expression. It consists of multiple elements that, when taken separately, drive an *hsp70-lacZ* test promoter in subsets of follicle cells: main body cells, centripetal cells, and border cells. Expression is not normally observed in border cells and possibly not in centripetal cells. It would be interesting to determine if YP expression in some of the subpatterns observed was sufficient to correct a corresponding YP mutation.

These studies suggest that YP expression is controlled by transcription factor binding to a series of sites associated with diverse spatial patterns of expression.

Three chorion genes, *s15*, *s36*, and *s38*, have been subject to detailed study of their *cis*-regulatory sequences controlling transcription (Wakimoto et al. 1986; Mariani et al. 1988; Romano et al. 1988; Tolias and Kafatos 1990). These studies accurately measured temporal and spatial control sequences; however, in many cases, the quantitative sufficiency of the control sequences was uncertain due to position effects. All three genes contained *cis*-regulatory sequences that extended only a few hundred base pairs upstream of the transcription start. The *s15* gene lost its normal restriction to S14 expression when a small upstream region was deleted, suggesting that negative regulation plays an important role in precisely timing gene expression (Mariani et al. 1988). Attempts to determine the functional capacity of *s36* genes driven by the various enhancers have not been attempted, although the existence of a specific mutation (*cor-36*) makes such experiments feasible.

Spatial regulation of chorion genes showed some of the same complexities as YP1 and YP2 regulation in follicle cells (Tolias and Kafatos 1990). An 84-bp proximal regulatory element (PRR) from position -49 to -132 drove strong expression in the follicle cells from an *hsp70-lacZ* promoter. Fusion genes were expressed early in dorsal cells that will lay down the bases of the dorsal appendages and also in a small group of follicle cells at the posterior end of the oocyte. By stage 12, expression spread to the remaining follicle cells over the oocyte. When the PRR was split in two at position -93, the frequency of position effects increased and average staining intensity decreased. However, different portions of the PRR tended to drive expression in either anterior or posterior cap follicle cells. The staining patterns were sensitive to changes in position and orientation, particularly with respect to expression in anterior cells. A region upstream of the PRR drove expression exclusively in dorsal appendages, although at a quantitatively low level.

These observations suggest a working model of transcriptional regulation in the follicular epithelium during oogenesis. The follicular epithelium has differentiated terminal cells as early as stage 1 or 2; a larger group of posterior cells is apparent almost as early, and dorsal-specific cells have been marked at least by stage 10A. Spatially specific transcription factors cannot ultimately explain the spatial patterns seen (what regulates their expression?). Cell-cell signaling systems are therefore likely to set up and maintain these informational groupings of cells. In response to signaling along these coordinate systems, posttranslational modifications (e.g., phosphorylation?) of relatively ubiquitous transcription factors would modify their interaction with other factors or their ability to compete for binding sites within enhancer regions. Thus, transcription patterns would follow the same spatial patterns as the signaling systems. However, combinatorial interactions along the multiple factors would allow more specialized patterns to be programmed. Uniform expression throughout the follicular epithelium might represent such a "complex" pattern.

Progress has been reported in identifying genes that regulate follicle cell genes during oogenesis. Two transcription factors, CF-1 and CF-2, specifically bind to *cis*-regulatory sequences upstream of the *s15* chorion gene (Shea et al. 1990). CF-1 binds specifically to the conserved chorion box sequence TCACGT located upstream of all *Drosophila* chorion genes and corresponds to the prod-

uct of the *ultraspiracle (usp)* (2C) gene (Henrich et al. 1990; Oro et al. 1990). Additional studies will be required to verify that *usp* actually functions to regulate chorion gene expression in follicle cells. If so, the structural similarity between the protein encoded by *usp* and the vertebrate retinoid receptor protein would suggest a role for a ligand in generating temporal and/or spatial expression patterns. Another putative *trans*-regulatory factor, YPF1, which binds to a 31-bp protein-coding sequence 148 bp downstream from the transcription start site, was identified and purified as a binding activity in Kc cells (Mitsis and Wensink 1989a,b). YPF1 activity first appears late in oogenesis (stages 11–14), so its significance in YP gene regulation remains uncertain.

I. Oocyte Construction

1. Component Storage

The oocyte cytoplasm stores large numbers of gene products for use during embryonic development. The great majority of these do not play a crucial role until after oogenesis is complete. However, it is necessary for developing egg chambers to synthesize appropriate amounts of these products at the appropriate time and to transport them from the nurse cells into the oocyte. Many gene products destined for the oocyte appear to be made in large amounts during stage 10B. Many of the substances stored in the oocyte probably also require special packaging to ensure that they are preserved and released at the appropriate time. Nuclear proteins may initially accumulate in the germinal vesicle (Smith and Fisher 1989; Ait-Ahmed et al. 1992; Segalat et al. 1992). However, when the germinal vesicle breaks down during stage 13, these proteins are released into the cytoplasm.

Storing structural and messenger RNAs for later use is a general problem faced by eggs. Under ideal circumstances, *Drosophila* eggs are immediately fertilized and embryonic development is initiated, so that extended periods of storage are not required. In such eggs, there is little decline in oocyte protein synthesis (Lovett and Goldstein 1977; Mermod and Crippa 1978). However, when stage-14 eggs are held, general protein synthesis decreases significantly. Unfortunately, it is not known how development is arrested in mature ovarian oocytes or whether the translation of specific mRNAs is controlled.

Genes encoding components of the protein synthetic machinery are likely to require special controls during oogenesis. In *Xenopus*, ribosomal protein production is regulated in oocytes and early embryos by controlling both the processing and stability of gene transcripts and the efficiency of utilization of mature mRNAs (for review, see Amaldi et al. 1989). Some ribosomal proteins may modulate the processing of their own transcripts by binding to intron sequences, unlike *Escherichia coli*, where they repress translation by binding to their mRNAs. In *Drosophila*, translation of at least some ribosomal proteins is specifically regulated during the first few hours of embryogenesis (Fruscoloni et al. 1983; Al-Atia et al. 1985). However, addition of extra ribosomal gene copies by transformation has not revealed the existence of homeostatic regulatory mechanisms (Tamate et al. 1990).

2. Pole Plasm Assembly

Drosophila eggs, like those of many insects, contain a distinctive pole plasm at their posterior ends that has long been implicated in germ-cell determination

(for review, see Mahowald 1992). Small organelles termed polar granules reside in the polar plasm in close association with mitochondria. They are taken up by pole cells and converted into a perinuclear nuage that is maintained throughout the life of germ-line cells. The protein encoded by *vasa* is a component of polar granules and persists in the perinuclear region of germ-line cells as predicted from earlier studies (Hay et al. 1988a,b; Lasko and Ashburner 1988, 1990). Polar granules are thought to be important in segregating and maintaining the germ line. For example, they may control the expression of germ-line-specific gene products. Karpen and Spradling (1990) proposed that many *Drosophila* somatic cells eliminate heterochromatic DNA sequences during development. Polar plasm might be part of the mechanisms that repress such elimination in the germ line. The localization of Tudor protein within mitochondria at the posterior pole provides additional evidence that mitochondria play some role in the pole plasm (McDonald et al. 1992).

Polar plasm can induce morphologically normal pole cells when micro-injected at the anterior end of early embryos and can rescue pole cell formation in eggs sterilized by posterior UV irradiation (see Mahowald and Kambyssellis 1980; Togashi et al. 1986). Kobayashi and Okada (1989) reported that hybrid-selected or in-vitro-transcribed large mitochondrial rRNA displayed activity in these assays, although no rationale for this result was provided.

Formation of functional polar plasm requires the activity of at least eight genes: *spire*, *cappuccino*, *oskar*, *staufen* (55A), *vasa*, *valois* (38B), *mago nashi* (57C), and *tudor* (Boswell and Mahowald 1985; Lehmann and Nüsslein-Volhard 1986; Manseau and Schüpbach 1989a; Lasko and Ashburner 1990; Boswell et al. 1991; Ephrussi et al. 1991; Kim Ha et al. 1991; St. Johnston et al. 1991). Molecular genetic studies of these loci have begun to define a pathway for polar granule assembly. Oskar and Staufen, as well as Vasa proteins, and osk RNA are all localized to the oocyte posterior during oogenesis and are likely to be granule components. *capu*, *osk*, *stau*, and *vasa* are each required for localization of Vasa protein at the posterior pole. In contrast, *valois* and *tudor* mutants inactivate pole plasm, despite normal or near normal *vasa* localization (see Lasko and Ashburner 1990). Other RNAs, including *nanos* mRNA (Ephrussi et al. 1991) and *cyclin B* mRNA (Whitfield et al. 1989; Dalby and Glover 1992) also appear to eventually become associated with polar granules.

3. Product Localization

Localization of specific molecules during oogenesis plays an important role in the anterior and posterior systems of positional information. For example, *bicoid* (*bcd*) mRNA is localized at the anterior end of the oocyte. Originally, a simple model for *bcd* mRNA localization was proposed in which uniformly distributed receptors bound the mRNA immediately after it was transported from the nurse cells (Macdonald and Struhl 1988). Part of the 3'-untranslated region was shown to be sufficient to confer anterior localization when fused to *lacZ* sequences. Alternatively, binding sites for *bcd* RNA might themselves be localized. *bcd* mRNA was observed to be nonrandomly distributed in nurse cells prior to transport, suggesting that localization might reflect some other aspect of cell polarity rather than simply ring canal location (St. Johnston et al. 1989). The *exuperantia* (57A) (Hazelrigg et al. 1990; Marcey et al. 1991) and *swallow* (5E) (Stephenson et al. 1988) genes are both required for *bcd* mRNA

localization. Studies of the latter product suggest that it encodes a cytoskeletal protein that might be involved in mRNA anchoring or transport (Stephenson et al. 1990).

Evidence of posterior localization is also first detected during vitellogenetic stages. Although *vasa* mRNA remains unlocalized, Vasa protein synthesized in the nurse cells begins to move into the oocyte and accumulate at the posterior pole at about stage 9. Not all of the protein moves to the posterior; the majority appears to remain unlocalized throughout the rest of the egg (Lasko and Ashburner 1990). Since microtubule inhibitors disrupt *bcd* mRNA localization (Pokrywka and Stephenson 1991), it may be transported along microtubules from the anterior of the egg. Bic-D protein, whose sequence suggests it associates by coiled-coil interactions (Suter et al. 1989; Wharton and Struhl 1989), and *swallow* may participate in this transport. Prelocalized proteins may anchor transported components once they reach the posterior. A number of enhancer-trap lines are specifically expressed only in posterior follicle cells.

V. CONCLUSION

Developmental genetic studies have begun to reveal a clearer picture of how oogenesis is accomplished in *Drosophila*. One emerging lesson appears to be the importance of the cytoskeleton. Transcription factors and signal transduction pathways certainly play vital roles in oogenesis, as in other developmental processes. However, systems of cytoplasmic transport appear to be crucial, from the time of oocyte determination in the germarium until the oocyte's components are fully loaded and localized. The cytoskeletal systems that coordinate shape changes and cell migrations appear to be equally important. Defects in these systems probably underlie many of the mutations that alter egg shape and structure. We can expect many more insights to come from studies of *Drosophila* oogenesis. It is hoped that the information and ideas collected here will assist workers who wish to enter this exciting field and stimulate further the now rapid pace at which it is advancing.

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