

Counting in oogenesis

Delbert A. Green II · Didem P. Sarikaya ·
Cassandra G. Extavour

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Abstract The determination of a precise number of cells within a structure and of a precise number of cellular structures within an organ is critical for correct development in animals and plants. However, relatively little is known about the molecular mechanisms that ensure that these numbers are achieved. We discuss counting mechanisms that operate during ovarian development and oogenesis.

Keywords Oogenesis · Germ-line stem cell · Morphogenesis · Ovary · *Drosophila*

Introduction

The study of the molecular genetic control of animal development has made great progress in two major areas: differentiation and proliferation. In many cases, a circuit-like network that regulates gene expression controls differentiation. Input to the circuit can be in the form of signals exchanged between cells or of a transfer of information through an intracellular cascade. The ultimate

goal is to ensure that the expression of genes dictating different cell fates is achieved in the appropriate cells and is prevented in others. Differentiation is not simply a binary switch: the control of the number of cells that acquire a specific fate is also a part of the differentiation process.

Great strides have also been made toward understanding proliferation, although here the problem is more complex. Whereas several mutations have been discovered that result in over- or under-proliferation of cell populations, the precise control of tissue-specific proliferation parameters is less well understood. Proliferation can be regulated either by non-autonomous control of cell cycle switches or by intrinsic control of a certain number of divisions and the molecular mechanisms involved can be markedly different in each case. This paper will deal with a third major problem, the problem of counting during development; this issue is at the interface of both differentiation and proliferation. Here, we use “counting” to refer to developmental decisions whereby specific numbers of groups of cells must adopt a certain fate or undergo a collective morphogenetic process to form a single structure.

The development of the reproductive system and the process of gametogenesis provide several clear instances of the precise genetic control of counting. Because the molecular genetic mechanisms of these processes are best understood in *Drosophila*, we will focus on two major instances of counting necessary for *Drosophila* oogenesis: the number of germ-line stem cells (GSCs) that undergo divisions and the number of structures that house the GSCs. Following an introduction to the structure of the *Drosophila* ovary, we will discuss examples of recent advances in understanding the method of counting in the GSC niche and conclude with the comparatively unexplored area of the genetic control of ovariole number determination.

Delbert A. Green II and Didem P. Sarikaya contributed equally to this work.

D. A. Green II
Department of Molecular and Cellular Biology,
Harvard University,
16 Divinity Avenue,
Cambridge, MA 02138, USA

D. P. Sarikaya · C. G. Extavour (✉)
Department of Organismic and Evolutionary Biology,
Harvard University,
16 Divinity Avenue,
Cambridge, MA 02138, USA
e-mail: extavour@oeb.harvard.edu

Structure of the fly ovary

In all insects, each of the paired ovaries (Fig. 1a) is partitioned into functional units called ovarioles. These act as assembly lines in which oogenesis proceeds, with progressively older oocytes being arranged from anterior to posterior (Fig. 1b). The anterior tip of each ovariole consists in a stack of somatic cells called terminal filament (TF) cells (Fig. 1c). Immediately posterior to the TF is the germarium where the process of oogenesis begins. The germarium contains GSCs, somatic stem cells, cap cells, gonias and early cysts, which ultimately give rise to the oocyte. Newly eclosed adult females possess two to three GSCs (Fig. 1c) tethered to somatic cells called cap cells, which secrete signals that maintain the stem cell population and are part of the stem cell niche. GSCs undergo asymmetric division, giving rise to one daughter cell that remains attached to the cap cells and another that is not in contact with the cap cells. The former cell remains a GSC by virtue of its contact with the niche, whereas the latter cell proceeds to gametogenesis. The oogenesis developmental program begins with four mitotic divisions called transit amplifying (TA) divisions, whose products are

surrounded by follicle cells, the daughters of somatic stem cells. Together, the 16 clonally related germ cells and their encapsulating follicle cells are called a cyst. Of the 15 cyst cells (called cystocytes) undergo rounds of endoreduplication and become polyploid nurse cells, which will contribute to oogenesis by providing the 16th cell, the future oocyte, with the mRNAs and proteins necessary for early embryonic patterning. All cells of a single cyst thus ultimately produce a single oocyte.

The next section examines the counting mechanisms controlling the differentiation and proliferation of the cells that produce differentiating gonias: the GSCs.

Counting GSCs

Oogenesis requires the regulation of the counting of two critical parameters: the number of GSC divisions and the number of GSCs themselves. The number of divisions that each GSC undergoes determines the number of eggs produced by the female and the reduction in number of GSC divisions over the animal's lifetime is responsible for decreased fecundity with age (Zhao et al. 2008). Compared

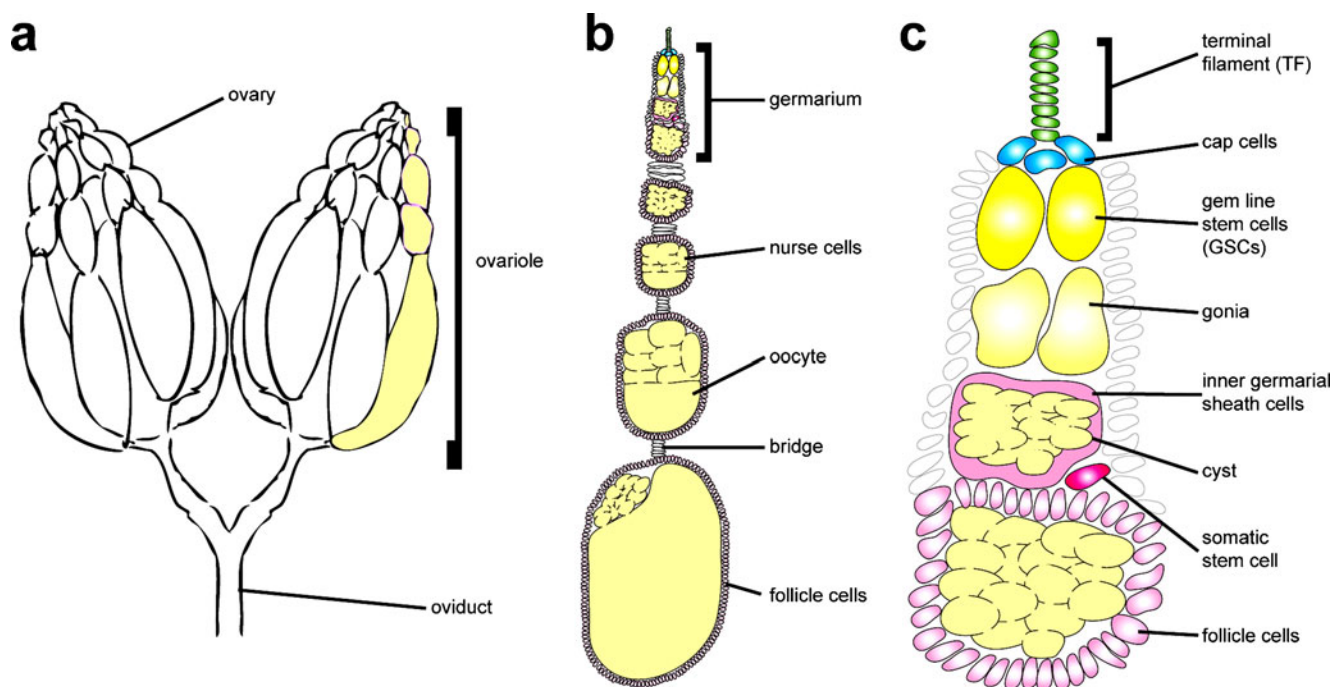


Fig. 1 Anatomy of an adult female ovary and oogenesis in *Drosophilids*. **a** Each of the two ovaries has individual oviducts that are connected by a common oviduct at the posterior. Each ovary is composed of several ovarioles. **b** Individual ovariole from a *Drosophilid* ovary. The germarium is at the anterior, followed by egg chambers at successively older stages of development. Egg chambers consist in one oocyte (at the posterior) and 15 interconnected nurse cells (at the

anterior). Each egg chamber is surrounded by a complement of follicle cells and connected by follicular bridge cells. **c** Germarium of an adult female ovary. Oogenesis begins at the anterior tip of the gonad (green terminal filament cells, blue cap cells, dark yellow germ-line stem cells [GSCs], light yellow gonias and differentiating gametogenic cells [cysts], dark pink follicle [somatic] stem cells, light pink follicle cells). Anterior is up

with 3-day-old females, the GSC division rate is reduced to 50% by 15 days and to 25% by day 40. This has a direct effect on fecundity, as egg production in these flies is reduced to 50% by 15 days and almost no eggs are produced by day 40. Flies carrying a mutation in the *Drosophila* insulin receptor homolog also have a reduced GSC division rate, suggesting that the insulin pathway and nutrition have an effect on the counting of GSC divisions (Hsu and Drummond-Barbosa 2009).

Similarly, both insulin signaling and aging also influence the number of GSCs in the germarium (LaFever and Drummond-Barbosa 2005; Zhao et al. 2008). The average GSC number per germarium decreases from 2–3 in young flies to 0–2 in old flies (Zhao et al. 2008). Interestingly, the counting of GSCs is closely tied to the interaction of these cells with the cap cells. When cap cells are induced to upregulate bone morphogenetic protein signaling, which is required for the maintenance of GSCs, older flies retain more GSCs than wild-type flies of the same age (Zhao et al. 2008). However, these flies only have a higher egg production rate as young adults, suggesting that fecundity is not simply a function of the absolute number of GSCs. Young flies with defective insulin signaling also show reduced GSC numbers, as reported by Hsu and Drummond-Barbosa (2009); although the authors have not reported the fecundity of these flies, the reduction of the stem cell population implies the cessation of de novo gametogenesis in those ovarioles, leading thereby to a reduction in fecundity. Whereas the factors determining their functionality are likely complex, the number of GSCs is clearly tightly regulated and the cap cell population appears to influence this instance of counting in the germarium. Given the central role of cap cells in the counting process, an understanding of the developmental origin, maintenance and roles of these cells is of important.

Cap cells are located posterior to the TF cells and anterior to the germ cells (Fig. 1c). Newly eclosed adults have four to five cap cells per niche. The role of cap cells in maintaining the appropriate numbers of GSCs has been illustrated by Hsu and Drummond-Barbosa (2009) who have found that, similar to the GSC number, the cap cell number declines as flies age. Moreover, flies that are mutant for the insulin receptor form fewer cap cells, which are lost more readily than in wild-type (Hsu and Drummond-Barbosa 2009). These flies then go on to lose GSCs because of insufficient signals from the cap cells. The counting of the cap cells is thus critical for the proper establishment and maintenance of the correct number of GSCs.

Cap cells originate from somatic cells adjacent to TF cells in the larval ovary, in a process that Song and colleagues (2007) have shown takes place at the larval-pupal transition and involves Notch signaling. When Notch signaling is ectopically activated in somatic cells surround-

ing GSCs, ectopic cap cells form by the recruitment of inner germarial sheath cells to a cap cell fate (Song et al. 2007). Similarly, the cap cell number also increases when GSCs are induced to overexpress the Notch ligand Delta (Ward et al. 2006). Not only do these flies have almost three times as many cap cells as young wild-type adults, the cap cell number also increases throughout adulthood. Taken together, these observations suggest that during development, many somatic cells surrounding GSCs are competent to differentiate into cap cells via Notch signaling but this differentiation normally takes place only in the four to five cells that are immediately anterior to germ cells in each ovariole.

Because the activation of canonical Notch signaling requires the Notch receptor to interact with its membrane-bound ligand, the receptor-expressing and ligand-expressing cells must be in physical contact with each other. During the normal establishment and maintenance of cap cells, it is therefore important to know the location of the expression of Notch and its receptors. At the larval-pupal transition, two cell types express Delta: GSCs and TF cells. Ward and colleagues (2006) have reported that Delta mutant GSCs are lost from the niche, suggesting that Delta expression plays a role in maintaining GSCs in the niche. However, in a subsequent study, Hsu and Drummond-Barbosa (2011) have observed neither a difference in GSC maintenance in niches containing Delta mutant GSCs compared with wild-type niches, nor a change in the cap cell number. These authors conclude that Notch ligands expressed in the GSCs do not play a role in cap cell counting. However, flies that do not have germ cells can form germaria without cap cells (Song et al. 2007).

TF cells also express Delta (Song et al. 2007). There are seven to ten TF cells per TF and TF cells can affect the cap cell number when the TF cell directly in contact with the cap cells lacks Delta activity (Hsu and Drummond-Barbosa 2011). Whereas the cap cell number is lower in these individuals, the number does not change significantly in adulthood, suggesting that Delta signaling from the TF plays a role during the organization of the organ during larval and pupal development but not in its maintenance during adulthood.

Currently, at least two important aspects of cap cell counting remain to be established: (1) the role of GSCs in cap cell formation and (2) the role of Delta signaling within cap cells. The current state of knowledge does not allow us to distinguish between a model in which the cap cells and GSCs regulate each other's numbers homeostatically (as has been observed for germ cells and intermingled cells in the larval ovary; Gilboa and Lehmann 2006) and one in which the correct number of cells is first established in one of these cell populations and subsequently determines the number of cells in the other population. To our

knowledge, no quantitative reports are available on how the loss of GSCs affects the cap cell number; this would be an interesting topic for future investigations and would help elucidate the role of signals from the GSC.

With respect to the second issue, the signaling via Delta is clearly important for cap cell number determination and maintenance but topics that remain to be resolved include the identification of the sources of the relevant signals and whether these sources are the same during ovarian development and throughout adult life. In larval and early pupal stages, Delta expression has been reported only in TF cells and GSCs (Song et al. 2007), suggesting that these cells induce competent somatic cells to become cap cells. However, the observations that adult cap cells are established and maintained in niches (1) with compromised GSC Delta function, (2) with compromised Delta function in the TF cell in contact with cap cells (Hsu and Drummond-Barbosa 2011), or (3) that lack GSCs entirely (Song et al. 2007) suggest that a non-GSC non-TF source of Delta might operate in adult niches. On finding that cap cells in normal adults express Delta and that some cap cells contact only each other and not TF cells or GSCs, Hsu and Drummond-Barbosa (2011) have proposed that Delta expression within the cap cell population provides sufficient signaling to establish and maintain correct cap cell numbers. To test this hypothesis, the determination of whether Delta expression can be detected in cap cell precursors or in cells adopting the cap cell fate during mid to late pupal stages would be of interest. Finally, although Notch signaling is the only pathway that has been specifically implicated in this process to date, additional signals might play a role in cap cell differentiation.

Counting during ovarian morphogenesis

Counting is integral for the precise construction of the gonad in the hexapods (Fig. 1a). Ovariole count is variable among insect species, ranging from one (in a Hawaiian fruit fly) to 1000 (in a beetle) per ovary (Büning 1979; Kambysellis and Heed 1971). However, the number is specific within species. For example, adult females of wild-type (Oregon R) *Drosophila melanogaster* have 18 ± 2 ovarioles at 25°C. The rate of egg production is constant per ovariole (maximum 2 eggs/ovariole per day in *D. melanogaster*) and independent of the number of ovarioles present in a single ovary (Cohet and David 1978). Egg production rate is positively correlated with the ovariole number, making it a strong determinant of reproductive capacity and hence of fitness (Cohet and David 1978).

The ovariole number is determined both environmentally and genetically. The majority of studies on this topic to date

have taken ecological, evolutionary, or quantitative genetic approaches. Studies of laboratory populations have revealed that an intraspecific maximum ovariole number is attained with optimal larval nutrition at intermediate temperatures (Delpuech et al. 1995; Thomas-Orillard 1984). In studies of natural populations of *D. melanogaster* and *D. simulans*, the ovariole number has been found to vary along a latitudinal cline, distinguishing populations within each species (Gibert et al. 2004). Both species show a similar clinal variation in the ovariole number, establishing that different species respond in similar ways to environmental influences. This suggests a common adaptive genetic basis of the trait. The best-studied Drosophilid example of ecological differences in the ovariole number is that of *D. sechellia*. This species occupies a specialized ecological niche in the Seychelles and possesses half the number of ovarioles as the generalist *D. melanogaster* (Louis and David 1986). The corresponding relative reduction in fecundity in *D. sechellia* (R'kha et al. 1997) might be offset by the unique advantage it holds in colonizing its niche: it has evolved the ability to metabolize a toxin produced by its host fruit, which is lethal to the competitor Drosophilid species (R'kha et al. 1991).

Quantitative genetic analyses of recombinant inbred and mutation accumulation lines of *D. melanogaster* have demonstrated significant segregating variation for the trait and have identified several autosomal effect loci (Wayne et al. 2001, 1997; Wayne and McIntyre 2002). Similarly, quantitative genetics approaches comparing *Drosophila* species (Coyne et al. 1991; Orgogozo et al. 2006) have validated older studies based on the coarse mapping of interspecies crosses (Thomas-Orillard 1976) and indicate that the principal loci that control number are found on chromosomes 2R and 3. However, specific genetic factors and an explanatory molecular genetic mechanism of counting have yet to be determined.

Consideration of ovarian development might shed new light on the ovariole number counting mechanism. In *D. melanogaster*, ovariole formation occurs in late larval life, beginning with the transformation of a group of anterior somatic cells into a specific number of organized stacks, the TFs (see above). Mutations that affect proper TF cell intercalation and recruitment (described in genes including *bric-a-brac*, *engrailed*, *hedgehog* and *twinstar*) lead to grossly abnormal ovarian morphology and adult female sterility, indicating that morphogenesis indirectly affects the ovariole number (Besse et al. 2005; Bolívar et al. 2006; Chen et al. 2001; Godt and Laski 1995). Counting has been addressed more specifically in the analysis of mutations in the *ecdysone receptor* and *ultraspiracle* genes, which encode nuclear co-receptors that regulate metamorphosis. Mutations in these genes cause mild defects in TF morphology and reduced ovariole numbers but adult

females are nonetheless fertile (Hodin and Riddiford 1998). TFs in these mutants are composed of more cells and form later in development than those in the wild-type, suggesting that ovariole counting is mediated by the ecdysone-dependent temporal control of TF cell morphogenesis.

In a final instance of counting within the ovary, each TF stack is composed of 7–10 cells (Godt and Laski 1995). The function and mechanism of this case of counting are unknown and largely unexplored. One hypothesis is that this specific cell number plays a structural role in early ovariole formation, as TF stacks serve as tracts along which apical cells travel and ensheath a pool of germ cells, thus delineating individual ovarioles. Alternatively, a specific TF cell number might be involved in maintaining the GSC niche, as TFs lie adjacent to the cap cells that form the GSC niche and express similar signaling factors. The genes that affect TF morphogenesis are good candidates for regulators of this counting mechanism.

Concluding remarks

Our understanding of the way that counting is regulated at molecular and developmental levels during gonadogenesis and gametogenesis is not yet complete. Even the few examples discussed here raise several specific questions that remain to be answered in future work. For example, what are the downstream targets of Notch signaling that induce cap cell fate? What are the targets of evolutionary change that result in the species-specific ovariole number? Given that little is known about the mechanistic regulation of this process, interspecies comparisons that reveal genes that have changed during evolution to cause changes in ovariole numbers between species might be fruitful starting points to identify candidates for advanced molecular genetic analysis in *D. melanogaster*. Finally, the number of ovarioles that have GSCs, the number of GSCs and their division rate and the number of TA divisions undergone by the gonial cells produced by GSCs are parameters that must be integrated during development. How are these decisions coordinated during development and throughout reproductive life? Further work on the molecular genetic basis of these processes is needed to provide answers to these questions.

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