

Drosophila Oocytes as a Model for Understanding Meiosis: An Educational Primer to Accompany “Corolla Is a Novel Protein That Contributes to the Architecture of the Synaptonemal Complex of *Drosophila*”

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SUMMARY Achieving a thorough understanding of the events and ramifications of meiosis is a common learning objective for undergraduate introductory biology, genetics, and cell biology courses. Meiosis is also one of the most challenging cellular processes for students to conceptualize. Connecting textbook descriptions of meiosis to current research in the field of genetics in a problem-based learning format may aid students’ understanding of this important biological concept. This primer seeks to assist students and instructors by providing an introductory framework upon which to integrate discussions of current meiosis research into traditional genetics or cell biology curriculum.

Related article in *GENETICS*: Collins, K. *et al.*, 2014 Corolla Is a Novel Protein That Contributes to the Architecture of the Synaptonemal Complex of *Drosophila*. *Genetics* **198**: 219–228.

THE purpose of meiosis is the faithful passage of genetic information from one generation to another. When meiosis functions properly, the integrity of the genome is preserved in the next generation, and viable offspring are produced. Meiotic defects, however, can result in sterility (failure to produce offspring) or developmental defects in offspring, often leading to premature death (Handel and Schimenti 2010). In fact, aneuploidy is one of the leading known causes of human congenital birth defects and miscarriage, as well as the leading impediment to successful pregnancies established using assisted reproductive technology (Nagaoka *et al.* 2012). Regulation of meiosis, therefore, is of critical importance to preserving a sexually reproducing species. Indeed, the molecular mechanisms that control the events of meiosis are highly conserved between different organisms,

underscoring the importance of meiotic regulation (Gerton and Hawley 2005).

Unlike mitosis, in which the end result is a diploid daughter cell with equal chromosome content and genetic identity to its mother, the end result of meiosis is a unique haploid daughter cell, distinct from both sets of parental DNA from which it descended (Gerton and Hawley 2005). In meiosis, a diploid cell will undergo one round of replication, forming sister chromatids. Following replication, meiosis can be simplified to three key steps: match chromosomes, lock chromosomes together, and move chromosomes to the right place (Gerton and Hawley 2005). Homologous pairs of sister chromatids are matched together in a process termed synapsis, which relies on the proper formation of a protein structure called the synaptonemal complex (SC) (Table 1). Association of the homologous chromosomes is essential for the exchange of genetic information; without proper matching, the remaining steps of meiosis are unlikely to proceed without error. Homologous chromosomes are locked together predominately by crossing over, wherein genetically programmed double-stranded breaks occur on the DNA and are resolved by homologous recombination. This also serves to exchange DNA from one

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Table 1 Abbreviations used in Collins et al. (2014)

Term	Abbreviation
Synaptonemal complex	SC
Double-strand breaks	DSB
Lateral element	LE
Central region	CR
Transverse filament	TF
Central element	CE
Structured illumination microscopy	SIM
Next generation sequencing	NGS
Ethane methyl sulfonate	EMS
Electron microscopy	EM

chromatid to the other, generating genetic diversity. Two divisions then physically move (disjoin) the DNA: homologous chromosomes separate in meiosis I, while sister chromatids separate in meiosis II. The segregation of DNA into four new cells (although in females of many organisms, only one of these cells will survive), shuffles the genetic material, producing a unique haploid genome.

Why Use *Drosophila* as a Model to Study Meiosis?

The fruitfly, *Drosophila melanogaster*, has long been a model system of choice for geneticists and cell biologists, largely due to the ease of their care and handling, short generation time (10 days at 25°), and large brood sizes (one female can lay >75 eggs per day). More recently, the wealth of genetic tools (particularly for cell-specific gene manipulation) and a fully sequenced and annotated genome have kept *Drosophila* at the forefront of modern genetics and cell biology research. Meiosis has been particularly well studied in *Drosophila* oocytes. Early experiments unequivocally demonstrated the chromosome theory of heredity, provided important observations regarding the role of chromosome sites (including the centromere and the telomere), and constructed the first meiotic map based on recombination frequency (reviewed in Lake and Hawley 2012).

One major reason for using *Drosophila* to study meiosis is the ease with which genetic mutants are created, maintained, and shared among members of the *Drosophila* research community. Mutants are the ultimate tool in a geneticist's or cell biologist's toolkit: they allow us to identify the biological role of a gene product within the context of a whole organism. Indeed, large-scale screens for meiotic mutants have helped propel our understanding of the molecular regulation of meiosis (Lake and Hawley 2012). *Drosophila* researchers also have an advantage in maintaining classical mutant alleles, due to the availability of a wide variety of balancer chromosomes (Figure 1). Balancers possess a large number of inverted sequences that act to suppress meiotic recombination. Most balancer chromosomes are homozygous lethal and carry visible dominant mutations that allow researchers to track the movement of the balancer from one generation to the next (Greenspan 2004; Chyb and Gompel 2013). These tools simplify genetic crosses, making it relatively easy to create double- and even triple-mutant fly lines in just a few generations. Additional tools to

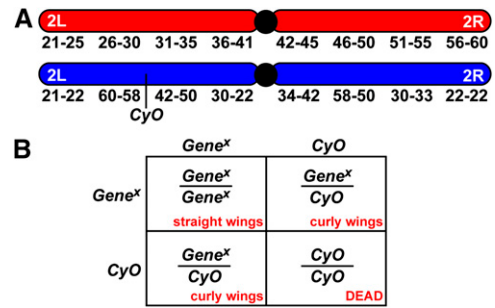


Figure 1 Balancer chromosomes allow for the maintenance of lethal mutant alleles. (A) Schematic representation of a balancer chromosome (blue) compared to a wild type chromosome (red). Numbers below represent regions of the chromosome. The balancer chromosome contains many inverted segments that effectively suppress meiotic recombination, and carries a visible dominant mutation (*CyO*). (B) Punnett square in which flies heterozygous for an allele of a gene of interest (*Gene^x*) and the balancer are mated. Phenotypes resulting from the presence or absence of the balancer are highlighted in red.

help associate mutant phenotypes with specific genetic loci, including chromosome duplications and deficiencies, are also available.

A second major reason that *Drosophila* ovaries are utilized as a model in which to study meiosis is the elegant, ordered simplicity by which oocyte development (oogenesis) proceeds. The *Drosophila* ovary consists of 15–20 ovarioles, each composed of progressively more developed follicles (Spradling 1993; Figure 2, A–C). Each follicle will not only harbor the oocyte, but also provide a microenvironment of support cells necessary for the development of the oocyte, the synthesis of the DNA and RNA stores that the early embryo will need for development postfertilization, and the production of the multilayered eggshell. By the end of oogenesis (at the posterior end of the ovariole), each follicle has matured into a large, yolk-filled oocyte.

Oocyte Development in *Drosophila*

The earliest events of *Drosophila* oogenesis occur at the anterior end of the ovariole, in a region known as the germarium (Spradling 1993; Lake and Hawley 2012; Figure 2D). Prior to adulthood, some primordial germ cells mature into germline stem cells, which reside in a protected microenvironment, or niche, at the very tip of the germarium (Xie 2013). Germline stem cells have not yet entered meiosis; instead, they undergo asymmetric mitotic divisions to create daughter cells that will undergo four subsequent mitotic divisions (Figure 2E). Because these mitotic divisions occur with incomplete cytokinesis, the resulting 16 germ cells, referred to as a germline cyst, remain interconnected by intracellular bridges, held open by rings of proteins (ring canals). Only one of the cyst cells will become the oocyte; the remaining 15 cells will become nurse cells. The oocyte is positioned into the posterior end of the developing cyst and remains in this position for the duration of oogenesis. Continued division of the germline stem cells helps to push developing germline cysts toward the posterior end of

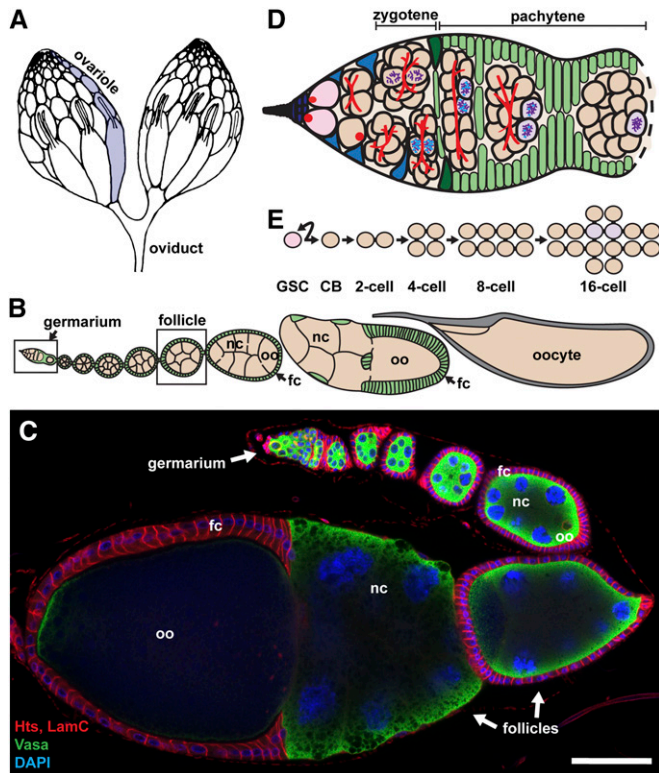


Figure 2 Schematic diagrams of the *Drosophila* ovary. (A and B) Each female fruitfly has a pair of ovaries (A), each consisting of 15–20 ovarioles, composed of progressively more developed follicles (B). (C) A 1- μ m optical cross-section of an ovariole (most mature stages have been removed); germ cells (green) labeled with antivasa, niche, and follicle cell membranes (red) labeled with anti-Hts and anti-LamC, and nuclei (blue) labeled with DAPI. fc, follicle cells; nc, nurse cells; oo, oocyte. Bar, 50 μ m. (D and E) The germarium (D) is composed of germline stem cells (pink) in their niche (terminal filament, cap, and escort cells; blue). Daughters of the germline stem cells (peach) divide to form a cyst (E); the pro-oocytes (gray) initiate SC formation in zygotene of prophase I. Follicle cells (light green), daughters of follicle stem cells (dark green), encapsulate the cyst at the posterior of the germarium.

the germarium. In the midsection of the germarium, the germline cyst encounters follicle cells, daughters of a second population of stem cells (Xie 2013). Follicle cells divide and surround the germline cyst; once an epithelial monolayer fully encapsulates the cyst, the follicle pinches off from the germarium (Spradling 1993). Follicles then progress through 14 distinct stages of maturation. Similar to the follicles beyond the germarium, cysts within the germarium are morphologically arranged in a predominately linear fashion in increasing stages of developmental maturity. This arrangement makes it easy to compare oocytes at different stages of meiosis within a single ovariole.

Like the familiar divisions of mitosis, meiosis is also divided into different stages that help describe the timing of meiotic events. Many students may be surprised to learn that, in most model organisms (and humans), meiosis in oocytes is characterized by a series of programmed pauses between different stages (Sen and Caiazza 2013). *Drosophila* oocytes are a good example: they remain in prophase I through most of oogenesis.

During late stages, oocytes are released to metaphase I, only to be arrested again until ovulation (Von Stetina and Orr-Weaver 2011). These pauses likely exist to accommodate the concurrent process of oocyte differentiation. It is well established that the events that occur during prophase I, including homolog pairing, synapsis, and crossover formation, are essential for the proper segregation of homologous chromosomes (Gerton and Hawley 2005). Prophase I is thus further subdivided (in *Drosophila* oocytes: leptotene, zygotene, and pachytene) to better understand the sequence of important regulatory events that occur during this prolonged period.

The work by Collins *et al.* (2014), is particularly focused on the earliest events of meiotic prophase I, during the zygotene and pachytene stages. It is during this time that the single oocyte is specified from within the 16-cell cyst, and the oocyte meiotic program is subsequently initiated. The oocyte remains in pachytene even after the follicle pinches off from the germarium (Lake and Hawley 2012).

The *Drosophila* Oocyte Synaptonemal Complex: Understanding Its Structure

Serial transmission electron microscopy studies from Carpenter in the 1970s elegantly demonstrated the tripartite structure of the SC in *Drosophila* and laid much of the groundwork upon which our knowledge of oocyte meiosis is based (Carpenter 1975; see also McKim *et al.* 2002 and Lake and Hawley 2012 and references therein). Similar to the sides of a ladder, two lateral elements form the junction between the central region of the SC and chromatin (Figure 3). Transverse filaments cross the central region perpendicular to the lateral elements, analogous to the rungs of the ladder. The central element runs down the middle of the central region, parallel to the lateral elements, like a rope thrown down the center of the ladder. Assembly of the SC is a dynamic process, and lateral elements and their precursors (referred to as axial elements) appear to be assembled first along the homologous chromosomes.

Analyses of genetic mutants have helped identify some of the proteins that compose the *Drosophila* SC (Lake and Hawley 2012 and references therein). Ord, Solo, and C(2)M are all components of the lateral elements and are required for early steps in SC assembly (Figure 3). Corona (Cona), a protein that localizes to the central element, and the transverse filament protein, C(3)G, are also required for SC formation, as well as the process of meiotic recombination. Prior to the work of Collins *et al.* (2014), C(3)G was the only known transverse filament protein in *Drosophila* (Lake and Hawley 2012).

Carpenter's electron microscopy studies of the SC (Carpenter 1975) present modern meiosis researchers with a fundamental problem: How do we deduce the molecular nature of a cytological structure? We know that the SC is critically important for meiosis, and we know at a nanometer resolution what the SC looks like. How do we identify the proteins that make up the structure? How can we determine which proteins

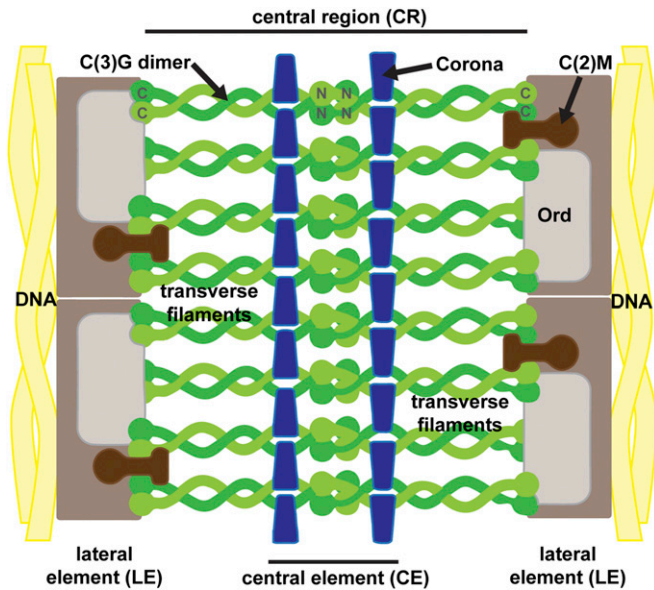


Figure 3 Schematic diagram of the synaptonemal complex, modeled after Lake and Hawley (2012). See text for description.

are associated with one another? What are the functions of these proteins?

Unpacking the Experiments

Collins *et al.* (2012) took a simple approach to the questions presented above. They knew that homologous chromosomes require the SC for proper chromosome segregation at meiosis I. If the SC is abnormally structured, or the adhesion of the chromosomes to the SC is impaired, then homologous chromosomes will prematurely come apart and segregate randomly, resulting in meiosis I nondisjunction. Since the SC is so crucial for proper chromosome segregation, they reasoned that a female fly harboring a mutation in a gene coding for a SC protein would produce offspring with very high levels of nondisjunction. They therefore generated a set of *Drosophila* mutants (more than 120,000!) to find genetic mutations that caused extremely high levels of nondisjunction (Collins *et al.* 2012). In the screen, flies must undergo a nondisjunction event (of an autosome) to survive (*i.e.*, “mutate my way or die”). Collins *et al.* (2012) identified several mutants in the screen, but chose to first characterize three that they discovered were alleles of a single gene (subsequently named *corolla*) through complementation analysis.

In the new research from the Hawley lab, Collins *et al.* (2014) investigate whether the protein encoded by *corolla* is truly a component of the SC, and, if so, how it fits into what is already known about the structure and molecular properties of the SC. They used super-resolution microscopy techniques to localize the protein within the SC and a classic molecular assay (yeast two-hybrid) to identify protein interactions between the new protein and other known SC components. Taken together, this work is a great example of how a combination of classical genetics, molecular biology, and cytological approaches can be used to probe the molecular regulation of important biological processes.

Phenotyping the mutants: Assaying for nondisjunction

Having found three mutants (originally named *mei-39¹*, *mei-39¹²⁹*, and *mei-39¹⁶⁶*) that displayed impaired meiotic phenotypes, the first step was to identify the gene harboring the mutagenized lesion. Collins *et al.* (2014) used next-generation, whole-genome sequencing to compare the genome of two of the mutant lines to a wild-type reference genome, and found that each mutant fly strain had mutations in a single uncharacterized gene, *CG8316*. The third mutant was subsequently shown to be an allele of *CG8316* as well by Sanger sequencing. Following their analysis of the protein encoded by *CG8316*, the Hawley lab named the gene *corolla* to reflect its cytological phenotype.

Previous studies demonstrated that in *Drosophila*, homologous chromosomes that undergo exchange (normal X chromosomes and autosomes) pair via a different mechanism than the non-exchange chromosomes (*i.e.*, the 4th chromosome; Gerton and Hawley 2005). A common feature between the two mechanisms, however, is the formation of the SC: if the SC is not formed properly, homologous chromosomes will segregate randomly, regardless of the mechanism by which they pair. Collins *et al.* (2014) therefore reasoned that if *Corolla* was a component of the SC, then *corolla* mutants should have high levels of both X and 4th chromosome nondisjunction (see figure 1B in Collins *et al.* 2014). Thus, in this context, levels of nondisjunction are being used as a “readout” for meiotic SC defects. While *Drosophila* genetics may appear complex in the materials and methods (Collins *et al.* 2014), the experiment is relatively easy, thanks to the facts that *Drosophila* are viable with certain sex chromosome aneuploidies and that sex is determined by X chromosome dosage (and not by the presence of a Y chromosome, as in humans). Collins *et al.* (2014) demonstrate that *corolla* mutants have remarkably elevated levels of X and 4th chromosome nondisjunction; in fact, the levels of nondisjunction on the X are nearly 50%, suggesting that these homologs essentially segregate at random. The nondisjunction phenotype of *corolla* mutants is completely rescued by the addition of a transgene that contains a wild-type copy of the *corolla* locus, indicating that the nondisjunction phenotype is specifically the result of mutating *corolla*.

Localizing the native protein: Immunofluorescence microscopy

While elevated nondisjunction in the *corolla* mutants suggests that this protein is associated with the SC, this evidence alone is not sufficient to make the conclusion that *Corolla* is one of the proteins that forms the SC structure seen in Carpenter’s electron microscopy images (Carpenter 1975). Collins *et al.* (2014) therefore used immunofluorescence microscopy to specifically visualize the protein in developing oocytes. Since native proteins are essentially without color, it is hard to look at a cell under a microscope and see the proteins themselves; we can see areas that are more or less dense, due to the refractive nature of the tissue in water, but we cannot see individual proteins. Immunofluorescence allows us to highlight native

proteins within intact tissue. Determining where in the cell (e.g., which organelle, which membrane) a particular protein is localized can give us a clue as to the function of that protein. Using immunofluorescence for the protein of interest, in combination with other fluorescent markers for particular cell landmarks (e.g., DNA), allows for more precise protein localization. Immunofluorescence can provide a framework upon which to visualize new uncharacterized proteins, cellular behavior, and genetic mutant phenotypes.

Localizing proteins via immunofluorescence requires at least two sets of antibodies. Primary antibodies are raised in different species (mouse, rabbit, rat, guinea pig, and chicken are the most common) and are selected for their high specificity for a protein of interest. While some primary antibodies are commercially available, Collins *et al.* (2014) had to first generate anti-Corolla antibodies to perform their immunofluorescence experiments. They produced an antibody to *Drosophila* Corolla by immunizing rabbits with a bacterially produced recombinant protein. Serum was collected from immunized rabbits and purified. With this new tool in hand, Collins *et al.* (2014) dissected and fixed *Drosophila* ovaries and applied rabbit anti-Corolla antibodies to the fixed tissue. They also applied mouse anti-C(3)G (a known component of the SC; Figure 3) antibodies. These antibodies were incubated for a given time, such that they could bind the respective native proteins expressed in the fixed tissue. Following a series of washes in a buffered detergent, they used secondary antibodies to detect the primary antibodies that bound to the ovary tissue. Secondary antibodies recognize the conserved or constant regions of antibodies specific to the species in which the primary antibody was generated. Secondary antibodies are frequently tagged with a fluorescent molecule (fluorophore) and must be raised in a different species than that used for the primary antibody (typically goat or donkey). Collins *et al.* (2014) used goat anti-rabbit antibodies labeled with a red fluorophore, and goat anti-mouse antibodies labeled with a green fluorophore, followed by super-resolution fluorescence microscopy, to experimentally visualize the location of Corolla in the context of the SC.

The use of structured illumination microscopy in these experiments is particularly exciting. The ability to visualize proteins in their native context is limited by the resolution at which microscopes can produce clear images. Carpenter was able to describe the SC in such detail (Carpenter 1975) because electron microscopy can detect cellular structure at subnanometer resolution (enough to see individual proteins, for example). The two-dimensional black and white transmission electron microscope image has rather limited utility, however, for visualizing *specific* proteins. The advent of super-resolution microscopes (like the structured illumination microscope) with enhanced resolving capacity allows researchers to combine the multiprotein localization capabilities of immunofluorescence with fine structural analysis in three dimensions. The work by Collins *et al.* (2014) demonstrates that structured illumination microscopy will be an important tool with which to further probe the molecular properties of cytological features.

Testing the physical interaction of Corolla and Corona: Yeast two-hybrid assays

The similar expression patterns of Corolla and Corona in the central region of the SC suggested that the two proteins might physically interact to help stabilize the structure (Figure 3). Collins *et al.* (2014) therefore used a yeast two-hybrid assay to test whether Corolla and Corona can bind to each other. Yeast two-hybrid analysis is based on the yeast transcription factor Gal4, which is split into two functional domains (an activating domain and a binding domain) required for binding DNA and activating transcription of target genes. A bait protein is fused to the binding domain, and a prey protein is fused to the activating domain. If the bait and prey proteins physically interact, the two domains are brought together and can initiate transcription of a reporter gene; typically, the reporter encodes proteins that permit biosynthesis of a certain combination of nutrients. Since the assay utilizes a mutant strain of yeast that cannot grow on media without these nutrients, yeast will only grow when the binding domain and activating domain (and thus, the proteins of interest) interact. While coimmunoprecipitation experiments could also have been used to test for a physical interaction between Corolla and Corona, these assays require a lot of starting material, and are not experimentally feasible for a protein interaction that happens in such a small number of cells.

Suggestions for Classroom Use

This primer seeks to bridge textbook descriptions of meiosis with current research investigating its molecular regulation. One potential approach for an entry-level course in Genetics would be to provide this Primer article concurrently with the Collins *et al.* 2014 article, following an in-depth discussion of the events of meiosis. To introduce students to experimental design and strategy, lectures could be accompanied by computer-based investigation of the genes discussed in these articles, using freely available resources such as FlyBase (www.flybase.org). For more advanced courses, additional in-depth reviews (McKim *et al.* 2002; Gerton and Hawley 2005; Lake and Hawley 2012) could be provided for background reading. Another recent research article exploring the mechanisms of meiotic chromosome pairing (Christophorou *et al.* 2013) could also be presented and compared within the context of the review literature. Upper-level courses in Cell Biology can also benefit from this Primer, as an entry point to understanding the cell biological principles that underlie oogenesis. The events of meiosis could be studied in parallel with germline differentiation, including topics such as stem cell maintenance, mitotic cell cycle control, cell-cell communication, and cell adhesion. For both types of courses, the relative simplicity of the experimental approach, as well as the plain language used to describe the experimental design and data, make the work of Collins *et al.* (2014) a good introduction to *Drosophila* research. Lastly, as *Drosophila* are one of the most accessible and user-friendly model organisms used in biological research,

lab course investigations could easily be added to a teaching module to reinforce concepts introduced by this research. A variety of excellent teaching modules (see Roote and Prokop 2013, for example), reviews (such as Spradling 1993; St Johnston 2002; McQuilton *et al.* 2012; St Johnston 2013; Hudson and Cooley 2014; Mohr *et al.* 2014), and handbooks (including Greenspan 2004; Ashburner *et al.* 2005; Chyb and Gompel 2013, among many others) are available to help students and instructors explore *Drosophila* in the classroom.

Questions for Review and Discussion

1. The authors indicate that they “will present evidence... that the novel protein Corolla is a transverse filament protein.” What key pieces of evidence support this conclusion?
2. Draw a diagram of the potential gametes produced by a wild-type and a nondisjunction mutant female. In figure 1B in Collins *et al.* (2014), how do the authors demonstrate that the nondisjunction phenotype in their *corolla* mutants is due specifically to a mutation in the *corolla* gene locus?
3. The *Drosophila* mutants described in the work by Collins *et al.* (2014) result in DNA-level changes to the *corolla* locus. One of these, *P{XP}corolla^{d01774}* is a *P*-element insertion into the *corolla* locus. What is a *P*-element? Why would the authors predict that this mutant would disrupt Corolla expression? Describe an additional experiment that would alter Corolla expression without making changes to the *corolla* locus.
4. In figure 1E in Collins *et al.* (2014), the authors compare the frequency of centromere identifier (CID) foci in wild-type and *corolla* mutant oocytes. Why is CID localization used as an assay for synaptonemal complex defects?
5. In figure 1F in Collins *et al.* (2014), the authors demonstrate that the number of DNA double-stranded breaks in *corolla* mutant oocytes is reduced. Why are *okra* mutant oocytes used as a control? Why is it useful to assay *corolla* mutants in an *okra*-deficient background?
6. In figure 2 in Collins *et al.* (2014), the authors use super-resolution microscopy to demonstrate that Corolla is expressed in the central region of the synaptonemal complex. Why did the authors choose to visualize C(3)G expression as a comparison? Which domain of the C(3)G protein do these antibodies recognize? What other antibodies could have been used to provide additional evidence supporting the localization of Corolla at the central region?
7. What is the technical difference in tissue preparation between panels A and B and C and D in figure 4 in Collins *et al.* (2014)? Do the authors get different results when they prepare the tissue in different ways? Why is this technical difference key to the interpretation of these results?
8. In their final experiment, Collins *et al.* (2014) utilize a *c(3)G* transgene that produces a mutant protein that cannot attach to the lateral elements. What *Drosophila* genetic tools are used to drive expression of the mutated version of C(3)G? How do the authors visualize the mutant C(3)G? Why do these results support the idea that

Corolla is a component of the central region? What would have been a good negative control for this experiment?

9. Collins *et al.* (2014) speculate that Corolla and C(3)G may interact via the predicted coiled-coil protein domains found in each protein. Design an experiment to test whether the coiled-coil domains of Corolla are important for its localization within the central region of the synaptonemal complex.
10. Collins *et al.* (2014) also hypothesize that Corolla could be the *Drosophila* ortholog of a *Caenorhabditis elegans* transverse filament protein called SYP-4. How could you test in *Drosophila* whether SYP-4 and Corolla are functionally conserved?

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