

The Formation of the Central Element of the Synaptonemal Complex May Occur by Multiple Mechanisms: The Roles of the N- and C-Terminal Domains of the *Drosophila* C(3)G Protein in Mediating Synapsis and Recombination

Jennifer K. Jeffress,^{*1} Scott L. Page,^{*2} Suzanne M. Royer,[†] Elizabeth D. Belden,^{*} Justin P. Blumenstiel,^{*} Lorinda K. Anderson[†] and R. Scott Hawley^{*1,3}

^{*}Stowers Institute for Medical Research, Kansas City, Missouri 64110, [†]Department of Biology, Colorado State University, Fort Collins, Colorado 80523 and [‡]Department of Physiology, University of Kansas School of Medicine, Kansas City, Kansas 66160

Manuscript received July 12, 2007

Accepted for publication September 27, 2007

ABSTRACT

In *Drosophila melanogaster* oocytes, the C(3)G protein comprises the transverse filaments (TFs) of the synaptonemal complex (SC). Like other TF proteins, such as Zip1p in yeast and SCP1 in mammals, C(3)G is composed of a central coiled-coil-rich domain flanked by N- and C-terminal globular domains. Here, we analyze in-frame deletions within the N- and C-terminal regions of C(3)G in *Drosophila* oocytes. As is the case for Zip1p, a C-terminal deletion of C(3)G fails to attach to the lateral elements of the SC. Instead, this C-terminal deletion protein forms a large cylindrical polycomplex structure. EM analysis of this structure reveals a polycomplex of concentric rings alternating dark and light bands. However, unlike both yeast and mammals, all three proteins deleted for N-terminal regions completely abolished both SC and polycomplex formation. Both the N- and C-terminal deletions significantly reduce or abolish meiotic recombination similarly to *c(3)G* null homozygotes. To explain these data, we propose that in *Drosophila* the N terminus, but not the C-terminal globular domain, of C(3)G is critical for the formation of antiparallel pairs of C(3)G homodimers that span the central region and thus for assembly of complete TFs, while the C terminus is required to affix these homodimers to the lateral elements.

THE synaptonemal complex (SC) is a protein lattice that connects paired homologous chromosomes in most meiotic systems. The functions of the SC, and/or proteins that compose the SC, include the maintenance or establishment of full homolog pairing, the conversion of double-strand breaks into mature reciprocal crossover events, and the control of the distribution of meiotic exchanges (for review see PAGE and HAWLEY 2004). Structurally, the SC resembles a railroad track. The two side rails of the SC, known as lateral elements (LEs), are interconnected by structures called transverse filaments (TFs). Proteins that form TFs have now been identified in several species. These include Zip1p in *Saccharomyces cerevisiae* (SYM *et al.* 1993), SCP1 in mammalian species (MEUWISSEN *et al.* 1992), C(3)G in *Drosophila melanogaster* (PAGE and HAWLEY 2001), SYP-1 and SYP-2 in *Caenorhabditis elegans* (MACQUEEN *et al.* 2002; COLAIÁCOVO *et al.* 2003), and ZYP1 in *Arabidopsis* (HIGGINS *et al.* 2005; OSMAN *et al.* 2006).

Although their primary amino acid sequences differ greatly, all TF proteins possess an extended coiled-coil-rich segment located in the center of the protein, flanked by largely globular domains at the N and C termini (Figure 1A). Immunolocalization of C(3)G, SCP1, and Zip1p by electron microscopy has elucidated the organization of these proteins within the SC (DOBSON *et al.* 1994; LIU *et al.* 1996; SCHMEKEL *et al.* 1996; DONG and ROEDER 2000; ANDERSON *et al.* 2005). As shown in Figure 1A, these observations suggest that individual TF proteins form parallel homodimers through their coiled-coil regions and that the N termini from opposing dimers interact, either directly or indirectly, through interactions with additional proteins in an antiparallel fashion to form tetramers. These tetramers become the TFs that span the width of the mature SC as the C termini interact with the LEs, while the N termini colocalize with the central element (CE) midway between the LEs.

Despite the apparent similarity in TF protein structure, data from yeast and mammals provide conflicting views of the roles of the N and C termini in the process of SC assembly. In yeast, the N-terminal globular domain of the Zip1 protein appears to be largely dispensable for SC formation, but the C-terminal domain is crucial for localizing and tethering TF proteins to the

¹Present address: Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

²Present address: Comparative Genomics Centre, James Cook University, Townsville, QLD 4811, Australia.

³Corresponding author: Stowers Institute for Medical Research, 1000 E. 50th St., Kansas City, MO 64110. E-mail: rsh@stowers-institute.org

chromosomes (TUNG and ROEDER 1998). Indeed, a deletion within the C-terminal domain of Zip1p results in the failure to bind to chromosomes but allows the aggregation of the protein into nuclear polycomplexes (PCs) (TUNG and ROEDER 1998). However, in a heterologous system where the wild-type rat SCP1 protein expressed in cultured somatic cells forms cytoplasmic PC structures but not canonical SC, a rather different set of results was obtained. ÖLLINGER *et al.* (2005) found that deletion of the C-terminal globular domain abolished PC formation, while a deletion of the N-terminal globular domain reduced the efficiency of PC formation but did not prevent this process altogether (ÖLLINGER *et al.* 2005).

To further understand the roles of the N- and C-terminal globular domains of TF proteins, we have designed a series of four transgenic constructs that express C(3)G with an in-frame deletion of all or part of either the N- or the C-terminal globular domain, along with full-length (FL) wild-type C(3)G controls. We have introduced the constructs into *Drosophila* and tested the deleted proteins for their ability to promote synapsis and recombination in meiosis *in vivo* compared to full-length C(3)G. In addition, we have analyzed the localization of the deleted C(3)G proteins to identify domains required for C(3)G function.

Here we report that deletion of the N terminus or of a segment at the N-terminal end of the coiled-coil domain completely ablates the ability of C(3)G to promote synapsis and recombination. C(3)G proteins lacking the C-terminal globular domain fail to localize along chromosomes and become concentrated within a cylindrical PC structure. The further analysis of these PCs by electron microscopy suggests that they consist of rolled sheets of self-assembled C(3)G proteins. On the basis of these data, we propose that the N and C termini of C(3)G play distinct roles in SC assembly, such that the N terminus and, in particular, a small segment of the coiled-coil region adjacent to the N terminus, is critical for the formation of antiparallel tetramers of C(3)G and that it is only such tetramers that can bind to the LEs. On the other hand, the C terminus is clearly necessary for the connection of such tetramers to the LEs of the SC.

MATERIALS AND METHODS

Transgene constructs: The *c(3)G* expression constructs were derived from the *c(3)G* cDNA LD07655. For each construct, either a wild-type or a modified cDNA sequence was amplified using primers carrying *NotI* and *Acl65I* restriction sites, digested with *NotI* and *Acl65I*, and ligated into *NotI*- and *Acl65I*-digested pUASP vector (RØRTH 1998) using standard procedures as outlined in detail in the supplemental Materials and Methods at <http://www.genetics.org/supplemental/>. Primer sequences are listed in supplemental Table 1. All constructs were verified by diagnostic restriction digests and DNA sequencing prior to creating transgenics. Transgenic flies were generated using standard methods (SPRADLING 1986). For some constructs,

generation of transgenic lines was performed by Genetics Services (Sudbury, MA). Transgene insertions were mapped to chromosomes by segregation analysis. Different insertions of the same construct are signified by a numbered suffix [e.g., *P{UASP-c(3)G^{FL}1}* and *P{UASP-c(3)G^{FL}3}* are two insertions of the *P{UASP-c(3)G^{FL}}* construct].

Expression patterns of the *nanos-GAL4::VP16*-driven constructs: As reviewed by SPRADLING (1993), *Drosophila* female meiosis begins within cysts of 16 interconnected germline cells. These cysts are formed when a germline stem cell, located at the anterior tip of the germarium, divides asymmetrically to produce a daughter cystoblast. In region 1 of the germarium, the cystoblast undergoes a series of four synchronous mitotic divisions with incomplete cytokinesis, resulting in 16 cells that are connected by intercellular bridges called ring canals. The ring canals serve as conduits for the transport of gene products from the outer cyst cells with fewer ring canals to the cell that will become the oocyte, which always has four ring canals. Meiotic prophase begins in the 16-cell cysts in region 2a of the germarium, where 4 cyst cells initially build an extensive SC. The meiotic stages of leptotene and zygotene occur in early region 2a, followed by pachytene, in which a full SC has formed (PAGE and HAWLEY 2001). The oocyte remains in pachytene from region 2a through region 3 in the germarium, while the other cells that initially build the SC revert to a nurse cell fate and lose their SCs. In region 2b of the germarium, the germline cysts flatten into a lens shape and are surrounded by a layer of somatic follicle cells, and in region 3 the cysts and their surrounding follicle cells bud off from the germarium to become egg chambers. Analysis of C(3)G localization showed that the SC is disassembled from the oocyte in a gradual fashion corresponding approximately to stages 2–8 of egg chamber development (PAGE and HAWLEY 2001). In the adult female germline, the *nanos-GAL4::VP16* driver (VAN DOREN *et al.* 1998) expresses a *UASP-lacZ* reporter gene in germline cells at all stages of oogenesis, starting with germarium region 1 (RØRTH 1998). Full-length C(3)G protein encoded by *UASP* constructs and driven by *nanos-GAL4::VP16* first appears in 16-cell cysts, like endogenous C(3)G, but tends to disappear earlier in meiotic prophase compared to endogenous C(3)G protein, starting around region 2b to region 3 of the germarium (data not shown). This was observed for both C(3)G^{FL} and C(3)G^{CFLAG}, while C(3)G^{Ndel}, C(3)G^{NCdel}, and C(3)G^{CC1del} disappeared abruptly after early region 2a, and C(3)G^{Cdel} could not be assessed due to its unusual localization (see RESULTS). The basis for the early disappearance of C(3)G^{FL} and C(3)G^{CFLAG} is unclear. Expression of both *UASP-lacZ* (RØRTH 1998) and *UASP-GFP* (data not shown) reporters driven by the *nanos-GAL4::VP16* driver shows an apparent decrease in region 3 and early egg chamber stages before increasing again in later stages. One possibility is that the maintenance of the SC requires the continual production of new C(3)G protein that is not maintained to a sufficient level in region 3 by *nanos-GAL4::VP16*. The robust rescue of the *c(3)G* mutant phenotype suggests that the role of C(3)G in recombination may be largely completed by the time of this early disappearance, however.

Measurement of meiotic recombination: C(3)G expression constructs were analyzed for their ability to rescue the meiotic recombination phenotype of *c(3)G⁶⁸* null mutants by genetic analysis for recombination on chromosome arm 2L (PAGE *et al.* 2000). In these assays, *y w nanos-GAL4::VP16/y w; net dpp^{ho} dp Sp b pr cn/UASP; c(3)G⁶⁸ e⁺ ca/c(3)G⁶⁸ e* (if insert was on the second chromosome) or *y w nanos-GAL4::VP16/w¹¹⁸ UASP; net dpp^{ho} dp Sp b pr cn/+; c(3)G⁶⁸ e⁺ ca/c(3)G⁶⁸ e* (if insert was on the X chromosome) females were crossed to *net dpp^{ho} dp b pr cn* males bearing a wild-type X chromosome. Genetic analyses were performed only on transgene insertions that mapped to the X

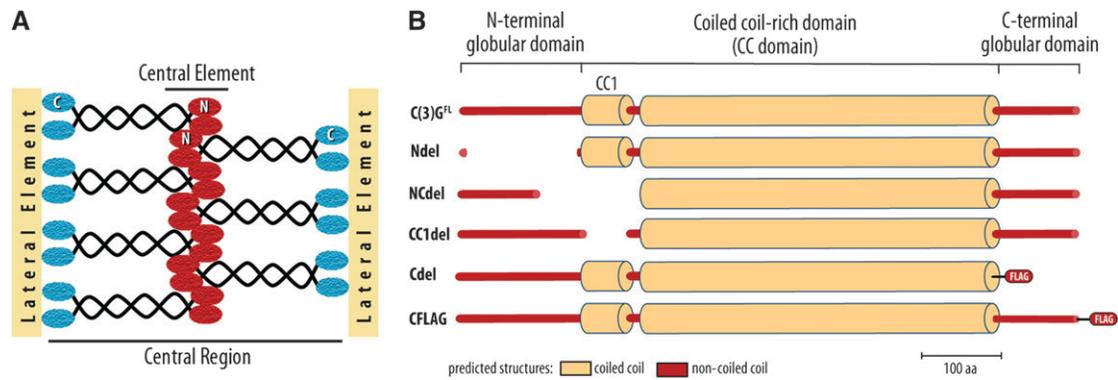


FIGURE 1.—(A) Model of the SC showing the orientation of the C(3)G protein. LEs, which line the length of homologous chromosomes, are interconnected across the central region of the SC by C(3)G homodimers, which make up the TFs. The C(3)G homodimers are oriented with the C-terminal domain (blue) aligned along the LEs and the N-terminal domain (red) interconnecting the C(3)G homodimers along the central element. (B) Full-length and deletion constructs of C(3)G. On the basis of protein secondary structure predictions for coiled-coil (tan cylinders) and non-coiled-coil (red lines) structure, C(3)G consists of a non-coiled-coil N-terminal domain, a central coiled-coil-rich region (CC) that includes a prominent short segment of coiled-coil (CC1), a non-coiled-coil gap near the N-terminal end, and a non-coiled-coil C-terminal domain. In-frame deletions of C(3)G are represented by open regions. FLAG epitopes are not drawn to scale.

or second chromosome. Complete genotypes used for meiotic recombination analysis are shown in supplemental Table 2 at <http://www.genetics.org/supplemental/>. To ensure that neither the *purple* nor *cinnabar* eye-color marker phenotypes were masked by incomplete expression of the *white* gene by the *w⁺* transgenes, recombination was scored only in female progeny (which received a wild-type X chromosome from their father). In this background, *dpt^{h^o}* showed variable penetrance and was not used in the recombination analysis.

Immunofluorescence and image analysis: For immunofluorescence of the full-length and mutant C(3)G proteins encoded by UASP constructs, expression was driven by *nanos-GAL4::VP16* in a *c(3)G⁶⁸* mutant background so that all of the detectable C(3)G protein originated from the construct. Ovarioles were stained for immunofluorescence (IF) as described by PAGE and HAWLEY (2001). Ovarioles were immunostained with a mixture of three mouse monoclonal anti-C(3)G antibodies (dilution 1:500) (ANDERSON *et al.* 2005), rabbit anti-Vasa (1:1000) (LASKO and ASHBURNER 1990), rabbit anti-Oo18 RNA-binding protein (ORB) (dilution 1:500) (NAVARRO *et al.* 2004), guinea pig anti-C(3)G (dilution 1:500) (PAGE and HAWLEY 2001), mouse anti-c-myc (dilution 1:500) (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-FLAG (1:500) (Sigma, St. Louis), or mouse anti-ORB 4H8 and 6H4 (dilution 1:30) (LANTZ *et al.* 1994), and DAPI. The generation of anti-C(3)G monoclonal antibodies was described by ANDERSON *et al.* (2005). Secondary antibodies Cy3 anti-guinea pig IgG (Jackson ImmunoResearch, West Grove, PA), Alexa 488 anti-mouse IgG, and Alexa 568 anti-rabbit IgG (Invitrogen, Carlsbad, CA) were used at a dilution of 1:500. Analysis of *c(3)G* mutants was performed using a DeltaVision RT restoration microscopy system (Applied Precision, Issaquah, WA) equipped with an Olympus IX70 inverted microscope and CoolSnap CCD camera. SoftWoRx v.2.5 software (Applied Precision) was used for image deconvolution and analysis. Figures were prepared with Adobe Photoshop. Images shown are single deconvolved optical sections.

Electron microscopy: Ovaries from female flies of the genotype *y w nanos-GAL4::VP16/y w ; P{UASP-c(3)G^{Cdel}}/4/+ ; c(3)G⁶⁸ e/c(3)G⁶⁸ e^{ca}* and Oregon-R controls were dissected 3–5 days post-eclosion and conventionally fixed using glutaraldehyde with osmium tetroxide postfixation as described by ANDERSON *et al.* (2005). Silver to pale gold (70–90 nm) serial

and semiserial sections were collected on formvar-coated copper slot grids and poststained with uranyl acetate and lead citrate. Sections were examined and photographed using an AEI 801 transmission electron microscope operated at 60 kV. Negatives were scanned and figures prepared with Adobe Photoshop. Three-dimensional reconstructions were prepared using the IMOD software package (KREMER *et al.* 1996).

RESULTS

Description of the full-length and deleted C(3)G expression constructs: As diagrammed in Figure 1B, previous analyses of secondary structure showed that the C(3)G protein contains a central coiled-coil-rich (CC) domain (amino acids 158–646) flanked by N- and C-terminal domains that are not predicted to form coiled-coils (PAGE and HAWLEY 2001, 2004). The CC domain contains four segments of coiled coils. The most N-terminal of these (CC1; amino acids 158–195) is separated from the other coiled-coil segments by a 28-amino-acid gap that lacks a predicted coiled-coil structure. The three remaining coiled-coil segments form a region (amino acids 224–646) in which only 4 of 423 amino acids score below the threshold for coiled coil (LUPAS *et al.* 1991) and possibly represent one long stretch of coiled coil that could form much of the transverse filament structure.

To identify functional domains of C(3)G, we have designed a FL wild-type C(3)G expression construct [*P{UASP-c(3)G^{FL}}*], a FL construct with a C-terminal FLAG epitope tag [*P{UASP-c(3)G^{CFLAG}}*], and a series of four constructs that express C(3)G proteins with in-frame deletions (Figure 1B). The in-frame deletion constructs are *P{UASP-c(3)G^{Ndel}}*, *P{UASP-c(3)G^{NCdel}}*, *P{UASP-c(3)G^{CC1del}}*, and *P{UASP-c(3)G^{Cdel}}*. *P{UASP-c(3)G^{Ndel}}* is an internal deletion that removes amino acids 4–153, removing most of the N-terminal globular

TABLE 1
Meiotic recombination in the presence of deleted C(3)G proteins

	UASP- <i>c(3)G</i> expression construct ^a												
	<i>y w</i>	<i>w</i> ¹¹¹⁸	<i>FL 3</i>	<i>CFLAG 85</i>	<i>Ndel 2</i>	<i>Ndel 5</i>	<i>NCdel 2</i>	<i>NCdel 4</i>	<i>CC1del 8</i>	<i>CC1del 9</i>	<i>Cdel 4</i>	<i>Cdel 7</i>	<i>c(3)G</i> ⁶⁸
	Map distances												
<i>net-dp</i>	11.05	11.63	9.67	3.93	0.00	0.00	0.00	0.00	0.10	0.22	0.86	0.00	0.00
<i>dp-b</i>	26.61	30.66	27.58	22.99	0.11	0.00	0.15	0.00	0.31	0.00	1.63	0.39	0.05
<i>b-pr</i>	4.72	9.86	9.09	16.11	0.11	0.24	0.15	0.26	0.21	0.55	0.77	0.26	0.17
<i>pr-cn</i>	1.07	1.32	2.52	5.11	0.11	0.00	0.00	0.26	0.00	0.00	0.38	0.00	0.02
Total	43.45	53.47	48.86	48.13	0.33	0.24	0.30	0.52	0.62	0.77	3.64	0.65	0.24
<i>n</i>	4490	4462	5159	1018	895	833	1319	1144	964	908	1046	777	4209
	Map relative to control ^b												
<i>net-dp</i>	1.143	1.203	1	0.406	0.000	0.000	0.000	0.000	0.010	0.023	0.219	0.000	0.000
<i>dp-b</i>	0.965	1.112	1	0.834	0.004	0.000	0.005	0.000	0.011	0.000	0.071	0.017	0.002
<i>b-pr</i>	0.519	1.085	1	1.772	0.012	0.026	0.017	0.029	0.023	0.061	0.048	0.016	0.019
<i>pr-cn</i>	0.425	0.524	1	2.028	0.044	0.000	0.000	0.103	0.000	0.000	0.074	0.000	0.008
Total	0.889	1.094	1	0.985	0.007	0.005	0.006	0.011	0.013	0.016	0.076	0.014	0.005
	Exchange rank frequencies												
<i>E</i> ₀	0.21	0.09	0.17	0.19	0.99	1.00	0.99	0.99	0.99	0.99	0.93	0.99	1.00
<i>E</i> ₁	0.71	0.75	0.70	0.66	0.01	0.00	0.01	0.01	0.01	0.02	0.07	0.01	0.00
<i>E</i> ₂	0.08	0.16	0.13	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>E</i> ₃	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

^aThe identity of the insertion tested in each column is shown by the number included with construct names.

^bMap distances are shown relative to $P\{UASP-c(3)G^{FL}\}3$, except for $P\{UASP-c(3)G^{Cdel}4$ and $P\{UASP-c(3)G^{Cdel}7$, which are shown relative to $P\{UASP-c(3)G^{CFLAG}85$.

domain but leaving the start codon intact. In $P\{UASP-c(3)G^{NCdel}\}$, amino acids 101–222 are deleted, removing part of the N-terminal globular domain, the CC1 coiled-coil segment, and most of the non-coiled-coil gap within the CC domain. $P\{UASP-c(3)G^{CC1del}\}$ is an internal deletion of amino acids 158–197 that specifically removes the CC1 coiled-coil segment. $P\{UASP-c(3)G^{Cdel}\}$ is a truncation of C(3)G that removes amino acids 651–744, including the entire C-terminal domain, with the addition of a FLAG epitope tag to the C terminus of the protein for immunolocalization studies.

Analysis of the full-length constructs: As controls for the study of the deleted C(3)G proteins, two types of full-length expression constructs, denoted $P\{UASP-c(3)G^{FL}\}$ and $P\{UASP-c(3)G^{CFLAG}\}$, were tested for their ability to build SC and facilitate recombination in flies homozygous for the $c(3)G^{68}$ null mutant. These constructs differ only by the addition of one copy of the FLAG epitope tag at the C terminus of the protein in $P\{UASP-c(3)G^{CFLAG}\}$. Because our anti-C(3)G antibodies bind the C terminus and the C-terminal portion of the coiled-coil domain (PAGE and HAWLEY 2001; ANDERSON *et al.* 2005), we tagged C(3)G^{Cdel} with a FLAG epitope at the C terminus to ensure that the C-terminal deletion could be analyzed by IF. Thus, the $P\{UASP-c(3)G^{CFLAG}\}$ construct serves as a control for any effects that result from the presence of the epitope tag on $P\{UASP-c(3)G^{Cdel}\}$.

We first determined whether full-length C(3)G expression in the female germline driven by *nanos-GAL4*:

VP16 could promote recombination in the absence of endogenous C(3)G. Recombination data for the left arm of chromosome 2 are presented in Table 1 for the $P\{UASP-c(3)G^{FL}\}$ and $P\{UASP-c(3)G^{CFLAG}\}$ constructs in a $c(3)G^{68}$ mutant background. Results for one insertion of each construct are shown. Both $P\{UASP-c(3)G^{FL}\}$ and $P\{UASP-c(3)G^{CFLAG}\}$ substantially rescue the defect in recombination observed in $c(3)G^{68}$ null mutants, although both constructs were susceptible to insertion-specific position effects. The frequency of recombination in the presence of the $P\{UASP-c(3)G^{FL}\}3$ insertion is elevated 200-fold compared to $c(3)G^{68}$ homozygotes lacking an expression construct, to 48.86 cM, slightly higher than that observed in a wild-type control with a similar genetic background (*y w*; 43.45 cM). In contrast, the $P\{UASP-c(3)G^{FL}\}1$ insertion displays a level of recombination that is only 70% of that observed in wild type (data not shown). We presume that this weaker ability of the $P\{UASP-c(3)G^{FL}\}1$ insertion to rescue the recombination defects observed in $c(3)G$ mutants is the result of an insertion-site position effect. Thus, we will rely on data for the $P\{UASP-c(3)G^{FL}\}3$ construct as our full-length control for the recombination analysis of the N-terminal deletions.

Similar results were obtained in oocytes carrying the $P\{UASP-c(3)G^{CFLAG}\}$ construct [denoted $P\{UASP-c(3)G^{CFLAG}85$ and $P\{UASP-c(3)G^{CFLAG}40$]. Females carrying the $P\{UASP-c(3)G^{CFLAG}85$ insertion in a $c(3)G^{68}$ mutant background displayed a level of recombination

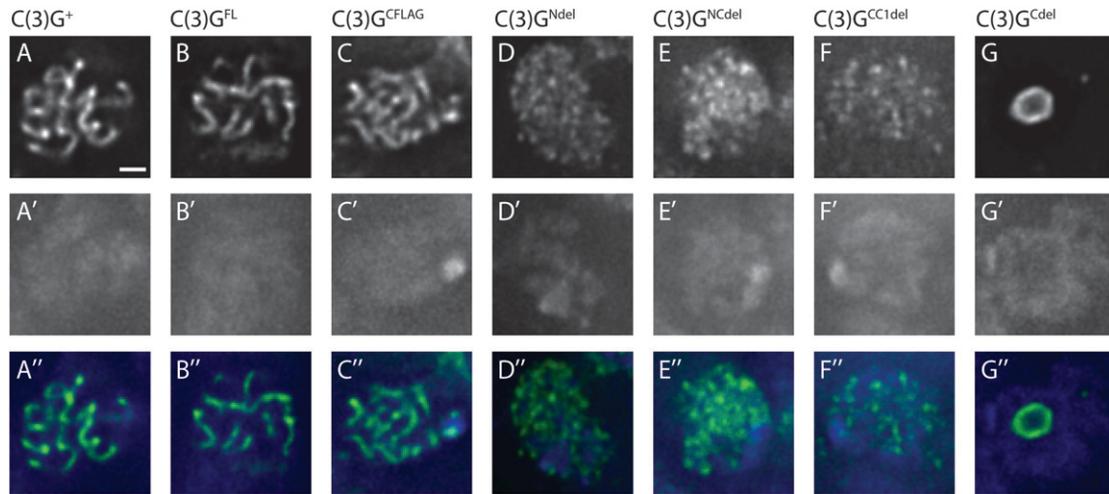


FIGURE 2.—IF images of *Drosophila* pro-oocyte nuclei expressing the indicated full-length or in-frame deletions of C(3)G stained with anti-C(3)G antibody. (A, A', and A'') *y w* (*c(3)G*⁺ control). (B, B', and B'') *P{UASP-c(3)G^{FL}}3*. (C, C', and C'') *P{UASP-c(3)G^{FLAG}}85*. (D, D', and D'') *P{UASP-c(3)G^{Ndel}}3*. (E, E', and E'') *P{UASP-c(3)G^{NCdel}}4*. (F, F', and F'') *P{UASP-c(3)G^{CC1del}}5*. (G, G', and G'') *P{UASP-c(3)G^{cdel}}4*. The C(3)G proteins were expressed in a *c(3)G⁶⁸* mutant background, so that localization of the antibody detects only the expression of the transgene-encoded protein in each case. (A–G) Anti-C(3)G immunofluorescence. (A'–G') Nuclear DNA stained with DAPI. (A''–G'') A merge of the anti-C(3)G IF (green) and DAPI (blue) images. Bar, 1 μ m.

for chromosome arm 2L, similar to the *P{UASP-c(3)G^{FL}}3* results and wild-type controls (Table 1). Thus, the FLAG-tagged C(3)G^{FLAG} protein is capable of rescuing the *c(3)G⁶⁸* defect in recombination back to normal levels, although the distribution of crossovers among the genetic intervals is slightly skewed in a polar fashion with more crossovers near the centromere and fewer crossovers in more telomeric intervals compared to controls. This type of altered distribution of recombination is also seen in cases of reduced C(3)G function, such as the *P{UASP-c(3)G^{FL}}1* insertion (data not shown) and certain other meiotic mutants (PAGE and HAWLEY 2005), and could mean either that the level of expression by *P{UASP-c(3)G^{FLAG}}85* is somewhat lower than that of wild type or that the C-terminal FLAG tag has a slight inhibitory effect on C(3)G function. However, as was the case for the *P{UASP-c(3)G^{FL}}1* insertion, a second insertion, *P{UASP-c(3)G^{FLAG}}40*, displayed a level of recombination that was only 45% of wild type (data not shown). Again, we presume that this weaker ability of the *P{UASP-c(3)G^{FLAG}}40* construct to rescue the recombination defects observed in *c(3)G* mutants is the result of a position effect. Because of its strong rescue of the *c(3)G⁶⁸* mutant phenotype, we will rely on data for the *P{UASP-c(3)G^{FLAG}}85* insertion as our full-length control for the analysis of the C-terminal deletion, C(3)G^{cdel}.

To determine the ability of the *nanos*-GAL4::VP16-expressed full-length C(3)G proteins to mediate synapsis, we determined their localization, as assayed by immunofluorescence in pro-oocytes using antibodies directed against C(3)G. In each case, the proteins were expressed in a *c(3)G⁶⁸* mutant background, so the only source of anti-C(3)G signal would originate from the transgene

construct. Localization of C(3)G^{FL} expressed from the *P{UASP-c(3)G^{FL}}3* insertion in gerarium region 2a is indistinguishable from that observed in wild type (Figure 2). Consistent with the incomplete rescue noted above, the localization of C(3)G^{FL} observed in region 2a in pro-oocytes carrying the *P{UASP-c(3)G^{FL}}1* insertion is often more discontinuous and less intensely stained than that observed either in wild type or in pro-oocytes carrying the *P{UASP-c(3)G^{FL}}3* insertion (data not shown). The FLAG-tagged C(3)G^{FLAG} expressed from the *P{UASP-c(3)G^{FLAG}}85* insertion showed normal localization (Figure 2), indicating that the epitope tag does not interfere with localization of the protein. In contrast, when expressed from *P{UASP-c(3)G^{FLAG}}40*, C(3)G^{FLAG} showed more discontinuous and less intense anti-C(3)G staining than was observed in pro-oocytes carrying *P{UASP-c(3)G^{FLAG}}85*, further suggesting that the difference in rescue is due to an insertion-specific position effect (data not shown). These results indicate that the full-length C(3)G proteins expressed from the *P{UASP-c(3)G^{FL}}3* and *P{UASP-c(3)G^{FLAG}}85* insertions localized normally and can be compared to the localization of internally deleted C(3)G proteins.

Deletions of N-terminal regions of C(3)G abolish both meiotic recombination and SC formation: The N termini of C(3)G molecules colocalize with the CE in the center of the mature SC and possibly serve to link C(3)G dimers together as TFs either directly or possibly by interacting with CE proteins analogous to the mammalian SYCE1, SYCE2, or TEX12 proteins (ANDERSON *et al.* 2005; COSTA *et al.* 2005; HAMER *et al.* 2006; BOLCUN-FILAS *et al.* 2007). To investigate the functional requirements of the N-terminal regions of C(3)G, we tested three constructs carrying in-frame dele-

tions [$P\{UASP-c(3)G^{Ndel}\}$, $P\{UASP-c(3)G^{NCdel}\}$, and $P\{UASP-c(3)G^{CC1del}\}$] for the abilities to facilitate recombination and support SC formation. Recombination was tested for two separate insertions of each construct in a $c(3)G^{68}$ null mutant background (Table 1). In contrast to the full-length $P\{UASP-c(3)G^{FL}\}3$ construct, none of the deleted C(3)G constructs rescued the recombination defect. Despite the presence of the $P\{UASP-c(3)G^{Ndel}\}$, $P\{UASP-c(3)G^{NCdel}\}$, or $P\{UASP-c(3)G^{CC1del}\}$ expression constructs, recombination remained at levels similar to that observed in oocytes homozygous for the $c(3)G^{68}$ mutant, indicating a complete failure to promote recombination. These results suggest that sequences within the N-terminal 222 amino acids of C(3)G are required for facilitating the formation of crossovers between homologs. This may not be a direct role in the enzymatic process of recombination, but might represent a function in which C(3)G participates that promotes recombination between homologous chromosomes. At least some of these sequences reside within the CC1 coiled-coil segment that is deleted in both C(3)G^{CC1del} and C(3)G^{NCdel}. However, additional sequences between amino acids 4 and 153 in the N-terminal domain are also required because the deletion in C(3)G^{Ndel} does not remove CC1 but similarly fails to rescue the recombination defect. This may represent a second region that is required or a larger region disrupted by all three deletions that encompasses both CC1 and a stretch of amino acids flanking CC1 on its N-terminal side.

Immunolocalization of the proteins expressed by the N-terminal deletion constructs $P\{UASP-c(3)G^{Ndel}\}$, $P\{UASP-c(3)G^{NCdel}\}$, and $P\{UASP-c(3)G^{CC1del}\}$ demonstrated that the recombination phenotype was not due to non-expression of the deleted proteins but revealed an altered pattern of localization compared to full-length C(3)G. Instead of localizing in thread-like signals (Figure 2, A–C), all three of the N-terminal deletion proteins display a similar spotty pattern of localization within the nucleus (Figure 2, D–F). The distribution of the deleted proteins within the nucleus was not uniform, however, and the signal tended to be brightest in areas of the nucleus in which DAPI staining of the DNA was most faint. These data suggest that the deleted C(3)G proteins tend to be located in areas that do not contain a high density of chromatin. Examples of this localization are shown for C(3)G^{Ndel} in Figure 2D and in supplemental Figure 1 (<http://www.genetics.org/supplemental/>). A putative nuclear localization signal (NLS) located at amino acids 55–61 either does not function as an NLS or is not required for C(3)G's ability to localize to the nucleus, as evidenced by the localization of C(3)G^{Ndel} within the nucleus. These observations suggest that a functional N terminus is required for normal SC formation and for normal levels of recombination. The same regions cited above as crucial for meiotic recombination are required for SC formation. This may be the result of two regions (CC1 and a portion of the N-terminal domain) that both

normally promote SC formation and recombination or a single region that includes sequences disrupted in C(3)G^{Ndel}, C(3)G^{NCdel}, and C(3)G^{CC1del}.

Unexpectedly, this study also produced evidence that a portion of the N-terminal domain may be necessary for regulating the timing of C(3)G protein accumulation. Full-length C(3)G^{FL} or C(3)G^{CFLAG} protein encoded by any of the four insertions tested was initially detected in 4 cells of each 16-cell cyst starting in germarium region 2a and persisted in the oocyte through late region 2b (supplemental Figure 2A at <http://www.genetics.org/supplemental/>). This was determined by co-immunostaining for cytoplasmic ORB protein, which appears contemporaneously with nuclear C(3)G protein in newly formed wild-type 16-cell cysts in region 2a (PAGE and HAWLEY 2001). However, for each of the two insertions of the $P\{UASP-c(3)G^{Ndel}\}$ and $P\{UASP-c(3)G^{NCdel}\}$ constructs tested, the C(3)G^{Ndel} and C(3)G^{NCdel} deletion proteins accumulated prior to region 2a and were not restricted to meiotic cells. Instead, C(3)G^{Ndel} and C(3)G^{NCdel} proteins were observed in region 1 during the mitotic cell divisions that form the 16-cell cysts and continued through region 2a before disappearing abruptly (supplemental Figure 2, B and C). Expression of C(3)G^{CC1del} protein was weaker than that of the C(3)G^{Ndel} and C(3)G^{NCdel} proteins. Immunofluorescence signals were sometimes visible in region 1 of germaria expressing C(3)G^{CC1del}, but were generally weaker than the signals in region 2a, making it difficult to conclude whether significant protein accumulation was occurring in region 1 (supplemental Figure 2D). Because the expression constructs are transcribed under the control of the *nanos*-GAL4::VP16 driver, which is active in germline cells throughout the germarium, these results indicate that the wild-type $c(3)G$ cDNA sequence contains information that controls the timing of C(3)G protein accumulation such that it is normally not detected until meiosis has initiated in region 2a. The deletions in the $P\{UASP-c(3)G^{Ndel}\}$ and $P\{UASP-c(3)G^{NCdel}\}$ constructs appear to disrupt this regulation. The deletions in the C(3)G^{Ndel} and C(3)G^{NCdel} proteins share amino acids 101–153, suggesting that a nucleic acid sequence within the region encoding residues 101–153 of wild-type C(3)G could be responsible for preventing the accumulation of C(3)G protein prior to the beginning of meiotic prophase. The mechanism of this regulation is unclear at present, but could involve transcriptional or translational repression or, alternatively, is an amino acid sequence that targets the protein for degradation in nonmeiotic cells.

Deletion of the C-terminal domain of C(3)G ablates meiotic recombination and results in polycomplex formation: Previous study of C(3)G localization suggested that the C-terminal globular domain of C(3)G may be involved in tethering the protein to the LEs (ANDERSON *et al.* 2005). To determine the importance of this domain for C(3)G function, we expressed C(3)G^{Cdel}, in which the C-terminal domain is deleted,

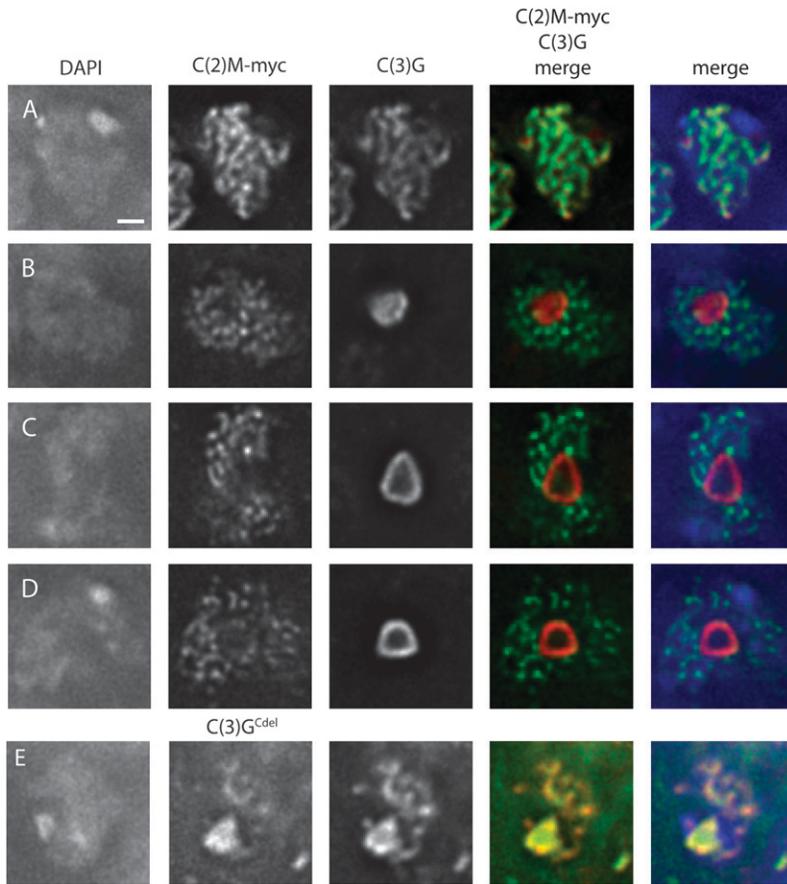


FIGURE 3.—IF images of *Drosophila* pro-oocytes of the genotype expressing the C(3)G^{Cdel} protein. (A) Single optical section of a *w*; *P*{*w*[+], *gc*(2)*M-myc*}*II.5*; *c*(3)⁺ pro-oocyte nucleus in which C(3)G^{Cdel} is not expressed. (B–D) Single optical sections of three different pro-oocyte nuclei of the genotype *y w*; *P*{*UASP-c*(3)G^{Cdel}}4/*P*{*w*[+], *gc*(2)*M-myc*}*II.5*; *c*(3)⁶⁸ *e/nos-GAL4::VP16 cu c*(3)G⁶⁸. A–D show the localization of C(2)M-myc, detected using anti-c-myc (green), and C(3)G, detected using anti-C(3)G (red) and DAPI (blue). (E) Single optical section of a *y w nanos-GAL4::VP16/y w*; *P*{*UASP-c*(3)G^{Cdel}}4/+; *c*(3)⁺ pro-oocyte nucleus, in which both C(3)G^{Cdel} and wild-type C(3)G are present. The images in E show the localization of C(3)G^{Cdel}, detected using anti-FLAG (green), and of C(3)G [both wild-type C(3)G and C(3)G^{Cdel}], detected using anti-C(3)G (red) and DAPI (blue). Bar, 1 μ m.

in a *c*(3)G⁶⁸ mutant background and tested the effect on recombination frequency and the ability of the protein to localize properly. As shown in Table 1, we tested two insertions of the *P*{*UASP-c*(3)G^{Cdel}} construct. Both of the insertions, *P*{*UASP-c*(3)G^{Cdel}}7 and *P*{*UASP-c*(3)G^{Cdel}}4, are unable to significantly rescue the meiotic recombination defect of *c*(3)G⁶⁸. The frequency of recombination in the presence of the *P*{*UASP-c*(3)G^{Cdel}}7 insertion resembled that of the *c*(3)G⁶⁸ mutant. However, *P*{*UASP-c*(3)G^{Cdel}}4 exhibited 3.64 cM of genetic map distance along chromosome arm 2L, a 15-fold increase in meiotic recombination relative to *c*(3)G⁶⁸. The higher level of recombination observed for *P*{*UASP-c*(3)G^{Cdel}}4 might reflect an SC-independent role for TF proteins in mediating recombination that leads to crossovers, a possibility previously raised by STORLAZZI *et al.* (1996). Although the cytological phenotype of the two insertions was indistinguishable (see below), the amount of protein expressed from the *P*{*UASP-c*(3)G^{Cdel}}7 insertion available to influence recombination could be altered by a position effect.

Immunolocalization of the C(3)G^{Cdel} deletion protein revealed striking differences from wild-type C(3)G. An identical phenotype was observed for both the *P*{*UASP-c*(3)G^{Cdel}}7 and *P*{*UASP-c*(3)G^{Cdel}}4 insertions, which were tested in a *c*(3)G⁶⁸ background. Rather than localizing in a thread-like pattern as expected for SCs (Figure 2, A–C), immunofluorescence of C(3)G^{Cdel} appeared as a

globular aggregate within germ-cell nuclei in regions 2a and 2b of the germarium (Figure 2G). In each nucleus, the antibody signal clustered in a single aggregate and did not show any thread-like staining. The aggregates varied in size, with the largest located in the pro-oocytes and the smaller aggregates in other cells of the 16-cell germline cyst (data not shown). This is similar to the manner in which wild-type C(3)G is concentrated in germline cyst cells with three or four ring canals and could represent a gradient resulting from transport of *c*(3)G protein or mRNA through the ring canals from outer cyst cells with fewer ring canals toward the oocyte. Especially apparent among the largest aggregates was their short cylindrical shape, which was deduced from nuclei containing aggregates oriented at varying angles and from projections of deconvolved image data. In some orientations, the aggregates showed a ring-like pattern of staining (Figure 2G, Figure 3, B–D), although at the resolution of fluorescence microscopy, it could not be determined whether this reflected true structural detail or was due to incomplete antibody penetration. The aggregates are likely to be PCs, which are assemblies of SC-like material that have been described in meiotic cells of many different species (ZICKLER and KLECKNER 1999). PCs are somewhat uncommon in *Drosophila*, but are occasionally observed (RASMUSSEN 1975; ANDERSON *et al.* 2005). In this study, the mutant C(3)G^{Cdel} protein

appears to form PCs in the nucleus rather than SC along chromosomes and does so in essentially every cell in which it is present.

The results for C(3)G^{Cdel} suggest that the C-terminal domain is required for C(3)G to attach to the LEs and that, in the absence of this domain, the protein assembles into PCs separately from the LEs. If this is the case, C(3)G^{Cdel} expression would be expected to have no effect on the assembly of LEs and on the localization of LE proteins. To investigate whether C(3)G^{Cdel} disrupted the assembly of LEs along homologous chromosomes, we determined the localization of the LE protein C(2)M (MANHEIM and MCKIM 2003) using a *P{gc(2)M-myc}* transgene (HEIDMANN *et al.* 2004). The localization of C(2)M-myc was unaffected by the expression of C(3)G^{Cdel} (Figure 3, B–D). C(2)M-myc formed thread-like signals, indicating that it was still able to localize along the chromosomes similarly to C(2)M localizing in the *c(3)G⁶⁸* mutant background. Furthermore, the C(2)M-myc protein did not appreciably colocalize with the C(3)G^{Cdel} aggregates. The observations that C(3)G^{Cdel} does not localize along chromosome axes and that C(2)M-myc does not associate with C(3)G^{Cdel} aggregates suggest that the PCs do not incorporate C(2)M and possibly no LE proteins at all, although LE proteins other than C(2)M have yet to be tested. We note that PCs that arise in the presence of certain yeast meiotic mutants contain LE-associated proteins such as Zip2p, Zip3p, and Zip4p in addition to Zip1p (TSUBOUCHI *et al.* 2006). The C(3)G^{Cdel} protein also does not have a great effect on normal C(3)G function. When expressed in a *c(3)G⁺* background, C(3)G^{Cdel} still formed aggregates, but wild-type C(3)G was able to form SC, and crossover frequencies were similar to wild-type controls (Figure 3E and data not shown). In pro-oocytes that expressed both C(3)G^{Cdel} and wild-type C(3)G, a faint anti-FLAG signal could be detected along the thread-like SC signals, indicating that the FLAG-tagged C(3)G^{Cdel} protein could localize along SCs (and thus LEs) formed predominantly by wild-type C(3)G, although none could be detected in the absence of wild-type C(3)G, showing that the C-terminal domain, or association with C(3)G protein with an intact C-terminal domain, is needed for association with LEs. The formation of the aggregates is largely independent of wild-type C(3)G, however, in that the aggregates were not inhibited in the *c(3)G⁺* background. The formation of the PCs by C(3)G^{Cdel} may represent a self-assembly process of the mutant protein, which is normally prevented in wild-type C(3)G by the C-terminal domain or possibly by the tethering of C(3)G to LEs.

Oocytes expressing C(3)G^{Cdel} form hollow, cylindrical PCs: Our fluorescence immunolocalization of the C(3)G^{Cdel} deletion protein suggested that it formed aggregates within nuclei, which may be similar to the PCs observed in yeast and other organisms (TUNG and

ROEDER 1998; ZICKLER and KLECKNER 1999). Serial sections of germaria from flies expressing the C(3)G^{Cdel} deletion protein in the absence of endogenous C(3)G were analyzed by electron microscopy to better characterize these PC structures. Germ-cell nuclei from these flies each contained a single PC and no SCs were observed, confirming our immunofluorescence results that the C(3)G^{Cdel} protein fails to form normal SC. Complete three-dimensional reconstruction of two PCs revealed hollow, barrel-shaped cylinders (Figure 4; supplemental Figure 1 at <http://www.genetics.org/supplemental/>). Partial and complete serial sections from 9 other PCs were consistent with this structure. Most of the PCs (9 of the 11 examined) were similar in size with a regular cylindrical structure (Figure 4D). These measured ~750 nm in length along the cylindrical axis and ~750 nm in diameter with the thickness of the walls measuring ~200–300 nm and the diameter of the central cavity ~150–160 nm. Overall, the 11 PCs ranged in size from 350 to 770 nm in length and from 600 to 900 nm in diameter.

The PCs were composed of alternating dark and light bands of approximately equal width oriented perpendicularly to the cylindrical axis, with most PCs having six to seven dark bands. Using measurements from regular (symmetrical) PCs sectioned parallel to the cylindrical axis, dark bands averaged 51 nm in width ($n = 89$) and light bands averaged 57 nm in width ($n = 72$). Some variation was apparent even among the regular PCs, which included one PC with a dark layer that extended across only part of the width of the PC while another PC had one dark layer that was much longer than all the others. Two of the PCs were irregular in shape and layer organization, forming, for example, an asymmetrical cylinder with dark bands oriented at varying angles with respect to each other and to the PC shape (see supplemental Figure 1 at <http://www.genetics.org/supplemental/> for three-dimensional reconstructions of a regular *vs.* an irregular PC). We observed lighter bands on the top and bottom ends of several of the cylindrical PCs, although the texture and similar shade of the surrounding nucleoplasm made visualization of these light bands difficult and therefore the light bands could have been present at the ends of all the PCs. Two PCs contacted the nuclear envelope, but most did not. We did not observe any recombination nodules in association with the PCs.

DISCUSSION

We have used in-frame deletions within the N- and C-terminal regions of C(3)G to analyze the functions of these domains in *Drosophila* oocytes. Unlike yeast and mammals, all three of the N-terminal deletion proteins tested [C(3)G^{Ndel}, C(3)G^{NCdel}, and C(3)G^{CC1del}] were completely incompetent in promoting both meiotic recombination and SC formation. Our findings suggest that both sequences within the CC1 coiled-coil segment

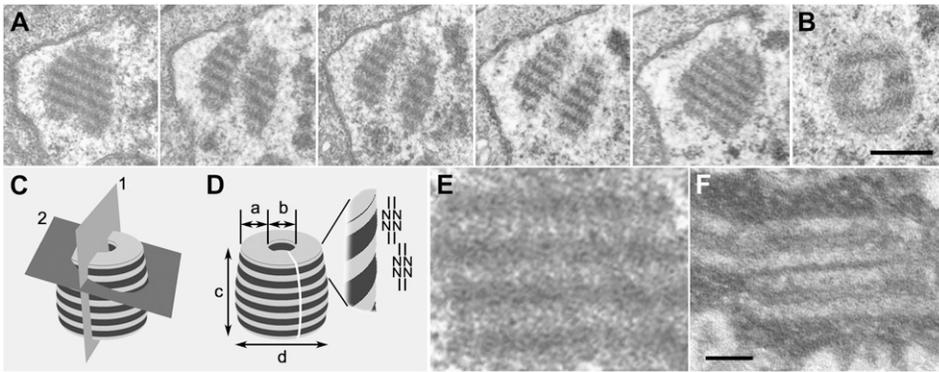


FIGURE 4.—Transmission electron micrographs and interpretive models of PCs from *Drosophila* females expressing the C(3)G^{C^{del}} deletion protein (A–E) compared to a more typical PC from *Drosophila* (F). (A) Series of five consecutive sections through a symmetrical cylindrical PC from a germlarium expressing C(3)G^{C^{del}}. The PC is sectioned parallel to the cylindrical axis, showing the pattern of alternating light and dark bands and the central space running from end to end of the PC. (B) Oblique

section through a second PC demonstrating the hole within the PC, the alternating light and dark bands, and the general circular shape of the entire PC. (C and D) Diagrams of the proposed model of PC structure as a thick-walled, hollow cylinder consisting of alternating dark and light layers. In C, two different planes of section are illustrated. Plane 1 corresponds to the plane of section of the micrographs in A, with the middle micrograph most closely corresponding to the diagrammed plane. Plane 2 is an oblique cut through a PC that is similar to the section shown in B. The diagram in D is labeled to indicate the dimensions of the PCs that were measured. The thickness of the walls (a) is ~200–300 nm and the diameter of the central hole (b) is ~150–160 nm. Both the length (c) and the diameter (d) are ~750 nm. A small section of the PC has been enlarged to show one possible orientation of the C(3)G^{C^{del}} proteins in spatial relationship to the PC layers. “N” represents the N-terminal globular domain and the vertical lines represent the coiled-coil-rich domain of the C(3)G^{C^{del}} proteins. The appearance of these unusual PCs can be explained if a large flat sheet of banded SC-like material interacts with itself (indicated here by a vertical white line through the model) to form a hollow cylinder. (E) Magnification of the far left section of the series in A, showing the alternate dark and light bands of the PC and the lack of narrow LE-like bands, which are found in typical PCs in *Drosophila*. (F) Example of a PC with typical structure in *Drosophila* (RASMUSSEN 1975; ANDERSON *et al.* 2005). The PC appears as stacked, closely apposed SCs with a narrow LE-like band between two central regions. Although E and F are both at the same magnification, the ovaries were not prepared in the same way, so direct physical measurements comparing the two cannot be made. However, comparison of E and F allows the differences in structure between typical PCs and the C(3)G^{C^{del}} PCs to be distinguished. Bar, 500 nm in A and B and 100 nm in E and F.

(amino acids 158–195) and sequences within the N-terminal domain (amino acids 4–153) are required for C(3)G to promote recombination and SC assembly, or possibly that a larger region spanning these two regions of the protein is required for these functions. It seems unlikely that the phenotypic defects in C(3)G^{CC1^{del}} are simply the result of a decreased total length of coiled coils within C(3)G. PAGE and HAWLEY (2001) showed that a larger in-frame deletion of coiled coils resulted in a milder phenotype than was observed for C(3)G^{CC1^{del}}. Given the arrangement of C(3)G proteins within the SC (Figure 1A; ANDERSON *et al.* 2005), these data may be explained if this region or regions close to the N terminus is critical for the formation of antiparallel pairs of C(3)G homodimers joined at or near their N-terminal ends [C(3)G tetramers] that span the central region of the SC as complete TFs. This tetramerization could be through direct binding or mediated by other proteins. C(3)G molecules that are unable to form these tetramers would be expected to be incapable of forming normal SC, and this might then result in a failure to promote meiotic recombination. We note, however, that the deleted C(3)G proteins observed by immunofluorescence do not appear to localize to chromosomes, suggesting that if this model is correct, tetramer formation may be necessary for chromosomal localization.

This observation that C(3)G^{N^{del}} could not mediate synapsis was initially surprising because in yeast the N-terminal globular domain of the Zip1 protein appears

to be largely dispensable for SC formation (TUNG and ROEDER 1998). In yeast expressing the Zip1-N1p protein, in which 80% of the N-terminal globular domain is deleted, homologous chromosomes undergo synapsis along their entire lengths, although they exhibit frequent synaptic and Zip1p-staining discontinuities that are not observed in wild type. However, the N-terminal domain of Zip1p may play a role in conjunction with part of the coiled-coil domain. When most of the N-terminal globular domain and an N-terminal 63-amino-acid portion of the coiled-coil domain are deleted in Zip1-NM2p, the extent of synapsis becomes highly variable and Zip1p staining becomes very punctate (TUNG and ROEDER 1998). Curiously, a deletion denoted Zip1-NM1p involving just the N-terminal portion of the coiled-coil domain showed a phenotype similar to Zip1-N1p. Thus, with some similarity to our results for C(3)G, it seems possible that in Zip1p the N-terminal domain and a portion of the adjacent coiled-coil domain are important for the ability to promote SC formation, yet these two regions might be somewhat internally redundant in the yeast Zip1 protein, in contrast to the requirement for both the N-terminal domain and CC1 segment of C(3)G indicated by the results for C(3)G^{N^{del}} and C(3)G^{CC1^{del}}.

The roles of the N-terminal domain and N-terminal coiled-coil regions of TF proteins were further explored in studies of ectopic SCP1 PC formation in mammalian cells performed by ÖLLINGER *et al.* (2005). These authors

found that an SCP1 protein lacking the N-terminal globular domain, but retaining an intact coiled-coil domain, formed long polymers similar to full-length SCP1 but did so with greatly reduced efficiency. The results led to the speculation that the N-terminal globular domain of SCP1 might play an important role in establishing contact between the N termini of SCP1 homodimers, but that once contact is made, stable associations can be maintained without the globular domain via contacts between the coiled-coil domains of opposing dimers (ÖLLINGER *et al.* 2005). This model would predict that the presence of the N-terminal domain, and presumably parts of the coiled-coil domain, affect the efficiency, but not the absolute ability, of TF proteins to assemble into higher-order structures. Consistent with this idea, we note that partial to nearly complete synapsis was occasionally observed even in yeast expressing Zip1-NM2p (TUNG and ROEDER 1998). Our results in *Drosophila*, however, suggest that the N-terminal globular domain and the adjacent C1 coiled-coil segment of C(3)G either are absolutely required or are crucial for the efficiency of higher-order assembly since the removal of either is sufficient to prevent the formation of normal SC.

Despite the differences in the behavior of N-terminal deletions of C(3)G compared to similar deletions of Zip1p and SCP1, there is good concordance between the behavior of deletions of the C-terminal globular domain in both *Drosophila* and yeast. Loss of the C-terminal globular domain of C(3)G in *Drosophila* oocytes prevents the localization of the deleted C(3)G^{Cdel} protein to the chromosomes, but allows its assembly into hollow, cylindrical PCs. Similarly, the yeast Zip1-C1 protein, which lacks a portion of the C-terminal domain, is unable to localize and tether polymerized TF proteins to the chromosomes (TUNG and ROEDER 1998). Expression of Zip1-C1p also results in the formation of large nuclear PCs that may be similar to those seen in *Drosophila*, although PCs also arise from the overexpression of wild-type Zip1p (SYM and ROEDER 1995). These results contrast sharply with results from studies of ectopically expressed SCP1, which causes cytoplasmic PC formation in a heterologous mitotic cell culture system (ÖLLINGER *et al.* 2005). In this system, expression of full-length SCP1 results in fibrous cytoplasmic PCs, but expression of SCP1 lacking the C-terminal globular domain fully blocks PC formation. Both the Zip1-C1p deletion protein in yeast and C(3)G^{Cdel} in *Drosophila* allowed the assembly of PCs, suggesting that PC formation in these organisms does not require an intact C-terminal domain, whereas in mammals, the full C-terminal domain of SCP1 may be needed for PC formation, or alternatively, the PCs studied by ÖLLINGER *et al.* (2005) differ in structure from the yeast and *Drosophila* PCs.

Our results demonstrate that a C-terminal deletion of C(3)G [C(3)G^{Cdel}] fails to attach to the LEs of the SC [as defined by the C(2)M protein] but rather aggregates

into large nuclear PCs. PCs in most organisms, including those previously characterized in *Drosophila*, usually look like closely apposed SCs that share LEs (Figure 4F; RASMUSSEN 1975). This also appears to be the case for PCs formed from Zip1p or SCP1 molecules (SYM and ROEDER 1995; DONG and ROEDER 2000; ÖLLINGER *et al.* 2005). However, electron microscopy (EM) analysis reveals that PCs from *Drosophila* females expressing C(3)G^{Cdel} are unusual in that no dark LE-like structure separates adjacent light bands, dark and light bands are similar in width, and the PCs usually form a hollow, cylindrical shape (Figure 4E). While PCs are aberrant structures that are variable in size, shape, and organization (reviewed by ZICKLER and KLECKNER 1999), to our knowledge, this is the first report of such a hollow, cylindrical type of banded PC. Although ring-like PCs were observed before (FILL 1978), they had no obvious banding and were considerably smaller in size, with a diameter of ≤ 150 nm.

The formation of PCs is generally thought to come about through an inherent capacity for SC proteins to self-assemble (ZICKLER and KLECKNER 1999; DONG and ROEDER 2000). Whether PCs arise as a normal physiological process or due to abnormal conditions, PC structures viewed by EM frequently suggest a regular organization of the constituent proteins. The PCs composed of C(3)G^{Cdel} proteins that we describe here usually have a hole that runs completely down the center of a generally cylindrical shape. One explanation for the presence of a hole within the PC is that the C(3)G^{Cdel} proteins initially self-assemble as a thick flat sheet that then bends and interacts with itself at each end to form a hollow cylinder. On the basis of previous immunolocalization of the N and C termini of C(3)G in normal *Drosophila* SCs (ANDERSON *et al.* 2005), the dark bands in the PCs may correspond to the N-terminal domains of C(3)G with the light bands corresponding to the coiled-coil portion of the proteins (Figure 4D). If this interpretation is correct, and assuming that binding of N termini to one another is complete, PCs should begin and end with light bands, which we sometimes observed. Also, the similar width of dark and light bands would indicate a significant overlap of coiled-coil segments in the light bands as compared to typical PCs (compare the relative proportions of PC structures in Figure 4, E and F). However, this SC-like organization is not the only kind that has been found among PCs. DONG and ROEDER (2000) reported two types of Zip1p-derived PCs and that the dark bands in PC “networks” labeled with antibodies to both N and C termini of Zip1p, indicating that these structures differ from normal SC. The dark bands of the C(3)G^{Cdel}-protein-containing PCs are relatively simple compared to the intricate structure of the CE in wild-type *Drosophila* oocytes. Thus, the possibility remains that the PCs resulting from C(3)G^{Cdel} expression could have an alternative internal organization of proteins as well.

In this study, we have analyzed in-frame deletions within the *Drosophila* TF protein C(3)G. Our results suggest that regions near the N and C termini of C(3)G play distinct roles in SC assembly. The N-terminal globular domain and an adjacent segment of the coiled-coil domain of C(3)G are needed for higher-order assembly of SC structure, probably through mediating the formation of antiparallel tetramers of C(3)G. The C-terminal globular domain also plays an important role in SC formation involving the binding of TFs to the LEs. The proteins identified as the constituents of TFs in diverse species differ so greatly in their primary amino acid sequences that they are not readily characterized as evolutionary homologs (MEUWISSEN *et al.* 1992; SYM *et al.* 1993; PAGE and HAWLEY 2001; MACQUEEN *et al.* 2002; COLAIÁCOVO *et al.* 2003; HIGGINS *et al.* 2005; OSMAN *et al.* 2006). Conversely, these proteins retain apparent similarities in their secondary structures and their functions in constructing morphologically conserved SC structures. The differences observed between the functions of the N- and C-terminal globular domains in flies, yeast, and mammals suggest that the sequence variation among TF proteins may correspond to different molecular mechanisms of TF assembly. Further studies will likely reveal additional similarities and differences among the molecular functions of TF proteins.

We thank C. Lehner for *Drosophila* stocks and C. Navarro, R. Lehmann, P. Lasko, and the Developmental Studies Hybridoma Bank for antibodies. This work was supported by the Stowers Institute for Medical Research. R.S.H. is an American Cancer Society Research Professor.

LITERATURE CITED

- ANDERSON, L. K., S. M. ROYER, S. L. PAGE, K. S. MCKIM, A. LAI *et al.*, 2005 Juxtaposition of C(2)M and the transverse filament protein C(3)G within the central region of *Drosophila* synaptonemal complex. *Proc. Natl. Acad. Sci. USA* **102**: 4482–4487.
- BOLCUN-FILAS, E., Y. COSTA, R. SPEED, M. TAGGART, R. BENAVENTE *et al.*, 2007 SYCE2 is required for synaptonemal complex assembly, double strand break repair, and homologous recombination. *J. Cell Biol.* **176**: 741–747.
- COLAIÁCOVO, M. P., A. J. MACQUEEN, E. MARTINEZ-PEREZ, K. McDONALD, A. ADAMO *et al.*, 2003 Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev. Cell* **5**: 463–474.
- COSTA, Y., R. SPEED, R. OLLINGER, M. ALSHEIMER, C. A. SEMPLE *et al.*, 2005 Two novel proteins recruited by synaptonemal complex protein 1 (SYCP1) are at the centre of meiosis. *J. Cell Sci.* **118**: 2755–2762.
- DOBSON, M. J., R. E. PEARLMAN, A. KARAIKAKIS, B. SPYROPOULOS and P. B. MOENS, 1994 Synaptonemal complex proteins: occurrence, epitope mapping and chromosome disjunction. *J. Cell Sci.* **107**: 2749–2760.
- DONG, H., and G. S. ROEDER, 2000 Organization of the yeast ZIP1 protein within the central region of the synaptonemal complex. *J. Cell Biol.* **148**: 417–426.
- FILL, A., 1978 Meiotic chromosome pairing and synaptonemal complex transformation in *Culex pipiens* oocytes. *Chromosoma* **69**: 381–395.
- HAMER, G., K. GELL, A. KOUZNETSOVA, I. NOVAK, R. BENAVENTE *et al.*, 2006 Characterization of a novel meiosis-specific protein within the central element of the synaptonemal complex. *J. Cell Sci.* **119**: 4025–4032.
- HEIDMANN, D., S. HORN, S. HEIDMANN, A. SCHLEIFFER, K. NASMYTH *et al.*, 2004 The *Drosophila* meiotic kleisin C(2)M functions before the meiotic divisions. *Chromosoma* **113**: 177–187.
- HIGGINS, J. D., E. SANCHEZ-MORAN, S. J. ARMSTRONG, G. H. JONES and F. C. FRANKLIN, 2005 The Arabidopsis synaptonemal complex protein ZYP1 is required for chromosome synapsis and normal fidelity of crossing over. *Genes Dev.* **19**: 2488–2500.
- KREMER, J. R., D. N. MASTRONARDE and J. R. MCINTOSH, 1996 Computer visualization of three-dimensional image data using IMOD. *J. Struct. Biol.* **116**: 71–76.
- LANTZ, V., J. S. CHANG, J. I. HORABIN, D. BOPP and P. SCHEDL, 1994 The *Drosophila orb* RNA binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev.* **8**: 598–613.
- LASKO, P. F., and M. ASHBURNER, 1990 Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. *Genes Dev.* **4**: 905–921.
- LIU, J. G., L. YUAN, E. BRUNDELL, B. BJORKROTH, B. DANEHOLT *et al.*, 1996 Localization of the N-terminus of SCP1 to the central element of the synaptonemal complex and evidence for direct interactions between the N-termini of SCP1 molecules organized head-to-head. *Exp. Cell Res.* **226**: 11–19.
- LUPAS, A., M. VAN DYKE and J. STOCK, 1991 Predicting coiled coils from protein sequences. *Science* **252**: 1162–1164.
- MACQUEEN, A. J., M. P. COLAIÁCOVO, K. McDONALD and A. M. VILLENEUVE, 2002 Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in *C. elegans*. *Genes Dev.* **16**: 2428–2442.
- MANHEIM, E. A., and K. S. MCKIM, 2003 The synaptonemal complex component C(2)M regulates meiotic crossing over in *Drosophila*. *Curr. Biol.* **13**: 276–285.
- MEUWISSEN, R. L., H. H. OFFENBERG, A. J. DIETRICH, A. RIESEWIJK, M. VAN IERSEL *et al.*, 1992 A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *EMBO J.* **11**: 5091–5100.
- NAVARRO, C., H. PUTHALAKATH, J. M. ADAMS, A. STRASSER and R. LEHMANN, 2004 Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nat. Cell Biol.* **6**: 427–435.
- ÖLLINGER, R., M. ALSHEIMER and R. BENAVENTE, 2005 Mammalian protein SCP1 forms synaptonemal complex-like structures in the absence of meiotic chromosomes. *Mol. Biol. Cell* **16**: 212–217.
- OSMAN, K., E. SANCHEZ-MORAN, J. D. HIGGINS, G. H. JONES and F. C. FRANKLIN, 2006 Chromosome synapsis in Arabidopsis: analysis of the transverse filament protein ZYP1 reveals novel functions for the synaptonemal complex. *Chromosoma* **115**: 212–219.
- PAGE, S. L., and R. S. HAWLEY, 2001 *c(3)G* encodes a *Drosophila* synaptonemal complex protein. *Genes Dev.* **15**: 3130–3143.
- PAGE, S. L., and R. S. HAWLEY, 2004 The genetics and molecular biology of the synaptonemal complex. *Annu. Rev. Cell Dev. Biol.* **20**: 525–558.
- PAGE, S. L., and R. S. HAWLEY, 2005 The *Drosophila* meiotic mutant *mei-352* is an allele of *khp3A* and reveals a role for a kinesin-like protein in crossover distribution. *Genetics* **170**: 1797–1807.
- PAGE, S. L., K. S. MCKIM, B. DENEEN, T. L. VAN HOOK and R. S. HAWLEY, 2000 Genetic studies of *mei-P26* reveal a link between the processes that control germ cell proliferation in both sexes and those that control meiotic exchange in *Drosophila*. *Genetics* **155**: 1757–1772.
- RASMUSSEN, S. W., 1975 Ultrastructural studies of meiosis in males and females of *c(3)G⁷* mutant of *Drosophila melanogaster* Meigen. *C. R. Trav. Lab. Carlsberg* **40**: 163–173.
- RØRTH, P., 1998 Gal4 in the *Drosophila* female germline. *Mech. Dev.* **78**: 113–118.
- SCHMEKEL, K., R. L. MEUWISSEN, A. J. DIETRICH, A. C. VINK, J. VAN MARLE *et al.*, 1996 Organization of SCP1 protein molecules within synaptonemal complexes of the rat. *Exp. Cell Res.* **226**: 20–30.
- SPRADLING, A. C., 1986 P element-mediated transformation, pp. 175–197 in *Drosophila: A Practical Approach*, edited by D. B. ROBERTS. IRL Press, Oxford.
- SPRADLING, A. C., 1993 Developmental genetics of oogenesis, pp. 1–70 in *The Development of Drosophila melanogaster*, edited by M. BATE and A. MARTINEZ ARIAS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- STORLAZZI, A., L. XU, A. SCHWACHA and N. KLECKNER, 1996 Synaptonemal complex (SC) component Zip1 plays a role in meiotic re-

- combination independent of SC polymerization along the chromosomes. *Proc. Natl. Acad. Sci. USA* **93**: 9043–9048.
- SYM, M., and G. S. ROEDER, 1995 Zip1-induced changes in synaptonemal complex structure and polycomplex assembly. *J. Cell Biol.* **128**: 455–466.
- SYM, M., J. ENGBRECHT and G. S. ROEDER, 1993 ZIP1 is a synaptonemal complex protein required for meiotic chromosome synapsis. *Cell* **72**: 365–378.
- TSUBOUCHI, T., H. ZHAO and G. S. ROEDER, 2006 The meiosis-specific Zip4 protein regulates crossover distribution by promoting synaptonemal complex formation together with Zip2. *Dev. Cell* **10**: 809–819.
- TUNG, K. S., and G. S. ROEDER, 1998 Meiotic chromosome morphology and behavior in *zip1* mutants of *Saccharomyces cerevisiae*. *Genetics* **149**: 817–832.
- VAN DOREN, M., A. L. WILLIAMSON and R. LEHMANN, 1998 Regulation of zygotic gene expression in *Drosophila* primordial germ cells. *Curr. Biol.* **8**: 243–246.
- ZICKLER, D., and N. KLECKNER, 1999 Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* **33**: 603–754.

Communicating editor: S. KEENEY