The CSN/COP9 Signalosome Regulates Synaptonemal Complex Assembly during Meiotic Prophase I of Caenorhabditis elegans

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Abstract
The synaptonemal complex (SC) is a conserved protein structure that holds homologous chromosome pairs together throughout much of meiotic prophase I. It is essential for the formation of crossovers, which are required for the proper segregation of chromosomes into gametes. The assembly of the SC is likely to be regulated by post-translational modifications. The CSN/COP9 signalosome has been shown to act in many pathways, mainly via the ubiquitin degradation/proteasome pathway. Here we examine the role of the CSN/COP9 signalosome in SC assembly in the model organism C. elegans. Our work shows that mutants in three subunits of the CSN/COP9 signalosome fail to properly assemble the SC. In these mutants, SC proteins aggregate, leading to a decrease in proper pairing between homologous chromosomes. The reduction in homolog pairing also results in an accumulation of recombination intermediates and defects in repair of meiotic DSBs to form the designated crossovers. The effect of the CSN/COP9 signalosome mutants on synopsis and crossover formation is due to increased neddylation, as reducing neddylation in these mutants can partially suppress their phenotypes. We also find a marked increase in apoptosis in csn mutants that specifically eliminates nuclei with aggregated SC proteins. csn mutants exhibit defects in germline proliferation, and an almost complete pachytene arrest due to an inability to activate the MAPK pathway. The work described here supports a previously unknown role for the CSN/COP9 signalosome in chromosome behavior during meiotic prophase I.

Introduction
The formation of haploid gametes is critical for reproduction in most eukaryotic organisms. Meiosis is the specialized cellular division leading to the formation of gametes, which in metazoans are eggs and sperm. Unlike mitosis, meiosis has one round of chromosome replication followed by two divisions: the first division is referred to as MI, in which homologous chromosomes segregate from each other, and the second division is referred to as MII, where sister chromatids segregate. It is essential that chromosome segregation during the divisions occurs correctly or an aberrant number of chromosomes will be present in the gametes, resulting in aneuploid eggs or sperm and consequently aneuploid or inviable offspring [1].

In meiotic prophase I, preceding the first division, homologous chromosomes pair, synapse, and form crossovers to recombine the genetic material. Crossovers and sister chromatid cohesion result in chiasmata, the visually detectable connections between homologous chromosomes observed in late prophase I. Chiasmata allow homologs to align properly at the metaphase plate during meiosis I and subsequently segregate to opposite poles [2]. All prophase I steps are highly regulated, ensuring that meiotic prophase proceeds correctly.

The synaptonemal complex (SC) is an evolutionarily conserved protein structure connecting pairs of homologous chromosomes during most prophase I stages and is required for formation of most crossovers [3]. Absent or improperly formed SC inhibits crossover formation, resulting in missegregation of chromosomes [4]. The SC is composed of lateral element proteins, which bind to the chromosomal axis of each homolog. The lateral element proteins are connected by the central region (CR) proteins, forming a physical link which holds homologous chromosomes together throughout most of meiotic prophase I [5–8]. In C. elegans, lateral element proteins include HTP-1/2, HTP-3, and HIM-3 [3–10]; there are four known CR proteins: SYP-1, SYP-2, SYP-3, and SYP-4 (collectively known as SYPs). The SYPs act in an interdependent manner: if one is missing, the CR does not form. The phenotypic consequences of mutations in all four SYPs are indistinguishable: lack of synopsis and failure to form crossovers [11–13].

In certain mutants, CR proteins can also assemble into aberrant SC-like structures that are non-functional and do not support...
Author Summary

Meiosis is a cellular division required for the formation of gametes, and therefore sexual reproduction. Accurate chromosome segregation is dependent on the formation of crossovers, the exchange of DNA between homologous chromosomes. A key process in the formation of crossovers is the assembly of the synaptonemal complex (SC) between homologs during prophase I. How functional SC structure forms is still not well understood. Here we identify CSN/COP9 signalosome complex as having a clear role in chromosome synopsis. In CSN/COP9 mutants, SC proteins aggregate and fail to properly assemble on homologous chromosomes. This leads to defects in homolog pairing, repair of meiotic DNA damage and crossover formation. The data in this paper suggest that the role of the CSN/COP9 signalosome is to prevent the aggregation of central region proteins during SC assembly.

crossover formation. In C. elegans, CR components are found to assemble between non-homologous chromosomes (non-homologous synapsis [7,9] or sisters [11,14]). CR proteins can also self-aggregate, forming polycomplexes (PCs). By electron microscopy, PCs are reminiscent of SC structures and in most cases, they are not associated with DNA [15]. Although PCs can contain multiple SC proteins, single components of the CR can form PCs without the aid of lateral element proteins [16]. PCs can be found in wild-type meiotic cells, when the SC assembles or disassembles, but these are small structures that are tightly regulated [17]. In some aberrant situations, large and persistent PCs are observed, indicating that in the absence of proper regulation CR proteins have a natural propensity to aggregate. These structures are found in tissue culture cells where CRs are expressed ectopically [18] and frequently found in yeast meiotic mutants [19]. In C. elegans, there are four examples for large and persistent PC-like structures (upon SC assembly [14,20,21] or disassembly [22]). The molecular mechanism leading to PC formation in these mutants is unknown.

Pathways regulating SC assembly to prevent PCs may be different between yeast meiosis and meiosis in other organisms. When recombination or SC assembly is perturbed, the yeast CR protein Zip1 readily forms PCs. On the contrary, none of the C. elegans CR proteins/SYPs aggregate when some SC proteins are missing or recombination fails [11–13,23] These observations raise the possibility that CR proteins self-aggregation (i.e., form PCs) is more tightly regulated in C. elegans meiosis.

In yeast and mouse, lateral element proteins have been shown to be post-translationally modified via sumoylation and phosphorylation which affects SC morphogenesis [24,25]. Proper SC assembly may also involve post-translational modifications of CR proteins. In C. elegans, it is not known if such mechanisms exist and how CR proteins are post-translationally modified.

The CSN/COP9 signalosome is a highly conserved protein complex involved in post-translational modifications, originally described in Arabidopsis as a repressor of photomorphogenesis [26]. The complex is comprised of eight subunits which are similar to the lid complex of the 26S proteasome [27,28]. Seven CSN/COP9 signalosome subunits have been identified in C. elegans. Five subunits (CSN-1,2,3,4, and 7) contain a PCI (proteasome, COP9 signalosome, initiation factor 3) domain and two (CSN-5 and CSN-6) contain MPN (Mpr1-Pad1-N-terminal) domains [29]. The PCI domains are thought to facilitate protein-protein interactions and may also have nucleic acid binding properties [29]. The CSN-5 MPN domain contains a JAMM (Jab1/Mpn/Cop9/Vps34) motif, which includes the metalloisopeptidase catalytic activity, which can cleave ubiquitin and ubiquitin-like post-translational modifiers (such as NED-8/Rub1) [30–33]. The CSN-6 MPN domain lacks the JAMM motif and thus the metalloisopeptidase activity [34,35]. The signalosome is involved in the regulation of protein function via multiple pathways, but most studies have been carried out in the context of ubiquitin pathway via the CULLIN-RING E3 ubiquitin ligases (CRLs) [27,32,36]. The signalosome, through deneddylation of the CRLs, down-regulates proteasome degradation and/or monoubiquitination of substrates [36–38]. This deneddylation activity occurs in the context of the signalosome holocomplex. The CSN/COP9 signalosome affects cell cycle, gene expression, and DNA damage repair, through mechanisms that do not necessarily involve CRLs [39–41]. The understanding of the role of the CSN/COP9 signalosome in meiosis is limited. In Drosophila, the CSN/COP9 signalosome is required for meiotic progression [42]. A recent study in Arabidopsis identified a role for neddylation in crossover distribution and SC assembly, but the CSN/COP9 signalosome was not yet examined in this context [43].

Null mutants of the CSN/COP9 signalosome generated in other model organisms (yeast and Drosophila) have shown that the loss of one subunit renders it inactive and leads to lethality [44–47]. CSN-5 (also known as Jab1) has been shown to act outside the holocomplex in such cellular activities as nuclear export, degradation, and protein stabilization [48,49]. The CSN-5 subunit of CSN/COP9 signalosome in C. elegans has also been implicated in muscle development [50], and the regulation of germline P-granule component, GLH-1, through interactions with KGB-1, a member of the JNK kinase family [51,52]. While CSN-5 RNAi has been shown to reduce the size of gonads in C. elegans [51,52], a role for CSN-5 in meiotic chromosome behavior has not been examined.

The work described here indicates a novel role for the CSN/COP9 signalosome in meiotic prophase I events that are key for the formation of functional gametes. Mutations in signalosome components specifically affected SC assembly and oocyte maturation. In csn mutants SYP-1 aggregated (PC-like structures) formed and persisted throughout meiotic prophase I. Additionally, we observed reduced chromosomal pairing throughout meiotic prophase as well as disruption in meiotic recombination and crossover formation. The defects in crossover formation were partially suppressed by reducing the levels of neddylation or ubiquitination. We also found an increase in apoptosis, likely due to the disruption of events earlier in pachytenes. Oocyte maturation was also disrupted, leading to a severe reduction in the number of oocytes in diakinesis, which rendered the worms sterile. Our working model is that the CSN/COP9 complex regulates SC morphogenesis by inhibiting SYP DNA-independent self-assembly. Without CSN/COP9 function SC morphogenesis is perturbed (leading to CR aggregate formation) as are subsequent downstream events (e.g., pairing and recombination) which are dependent on proper SC formation. Furthermore, the CSN signalosome affects oocyte maturation and permitting meiotic progression via MAPK/MPK-1 activation.

Results

csn mutants exhibit defects in SC morphogenesis and meiotic progression

We identified csn-5 as a gene involved in SC morphogenesis via an RNAi suppressor/enhancer screen of a mutant (akir-1) exhibiting aberrant SC aggregation. Previous studies utilizing RNAi methodology to examine the role of the CSN complex genes in C. elegans demonstrated that csn-5 was required for normal
gonad morphology. csn-5(RNAi) resulted in formation of short gonads and down-regulation of the P-granule component GLH-1 [51,52]. However, SC morphogenesis, chromosome dynamics, or meiotic recombination in meiotic prophase I were not examined in these studies. Here we focused our studies on the function of the CSN/COP9 signalosome in these meiotic processes.

Three deletion alleles were analyzed in this study: csn-2(tm2823), csn-5(ok1064) and csn-6(ok1604) (See also Materials and Methods). The csn-2(tm2823) allele is missing most of exon 3 which results in deletion of 28% of the coding region, including the PAM sub-domain [53] in the PCI domain (Figure 1A). The csn-5(ok1064) allele is missing exons 1, 2, and 3 which results in deletion of 64% of the coding region (including the MPN catalytic domain, Figure 1A). The csn-6(ok1064) allele is missing most of exons 1 and all of exon 2 which results in deletion of 43% of the coding region (including most of the MPN domain, Figure 1A). The csn-2(tm2823), csn-5(ok1064) and csn-6(ok1604) alleles will be referred here collectively as csn mutants.

In wild-type nuclei, SC assembly is initiated at the transition zone (leptotene/zygotene) when SC proteins load on chromosomes (Figure 1B); the SC is fully assembled at pachytene. SC disassembly is initiated at the end of pachytene and CR disassembly is complete by the end of diakinesis. To determine if SC morphogenesis was affected in csn mutants, we performed immunohistochemical analyses using antibodies against SYP-1, SYP-4, HIM-3, and HTP-3 [5,8,12,22] (Figure 1B,C; Figure S1 and S2). In all csn mutants, we observed smaller, morphologically different gonads compared to wild-type (Figure 1C), as previously published for csn-5(RNAi) [51,52]. The nuclei in the csn mutant gonads were unevenly spaced throughout the gonad. There also appeared to be no distinct rachis (central canal) as in wild-type worms. The chromosomes of csn mutants clustered to one side of the nucleus (a polarized organization) as found in the wild-type transition zone (leptotene/zygotene) nuclei; this is indicative of meiotic entry [54]. Unlike wild-type, in the csn mutants these polarized nuclei were found throughout the gonad intermixed with nuclei with a dispersed chromosomal organization. The persistence of polarized chromosomes has been observed previously in synopsis defective mutants [11]. In addition to the persistent polarized chromosome organization, we also determined the mitotic/meiotic boundary using antibodies for lateral element proteins, HIM-3 and HTP-3. Since these lateral element proteins localize to chromosomes axes upon the transition from mitosis to meiosis. This localization occurred concurrently with polarization of chromosomal organization and did not show any defects in the germline of csn mutants. These data indicate: 1) the transition from mitosis to meiosis took place in the csn mutants, and 2) the localization of lateral element proteins of the SC was not perturbed in csn mutants (Figure S1). Thus, although gonads are smaller in csn mutants and have fewer nuclei, meiotic entry has occurred and SC assembly has initiated.

In contrast to the pattern of localization of lateral element proteins in the csn mutants, the CR protein SYP-1 showed an aberrant pattern of localization. SYP-1 protein aggregates (PC-like structures) were found in the transition-like zone at the distal end of the gonad and through the late-pachytene-like zone in the proximal end of the gonad. These occurred in 100% of the gonads examined (wild-type n = 26, csn-2 n = 37, csn-5 n = 34, csn-6 n = 35; p<0.0005; Fisher’s Exact Test) for all csn mutants (Figure 1B). CR/SYP aggregates were 4 fold wider than a typical SC width of wild-type GC (0.22 μm ±0.23; n = 25, width of SC aggregate-csn-2 0.83 μm ±0.23, n = 70 and csn-5 0.86 μm ±0.31, n = 90 p<0.0001 Mann Whitney Test) and typically there was one aggregate per nucleus (csn-2 1.12, n = 62 and csn-5 1.13, n = 82).

While some nuclei contained a single SYP-1 aggregate with no additional SYP localization, most nuclei contained partially assembled linear SC (similar to that observed in wild-type gonads) in addition to the aggregate (detailed analysis below). As there are currently four SYP proteins, we examined the localization of SYP-4 in csn-5 mutants as well as GFP:SYP-3 in csn-2 and csn-5 mutants to identify if the aggregation defects were specific to SYP-1 or generally affect all the CR components. SYP-3 and SYP-4 also form persistent aggregates suggesting the defects observed in the csn mutants are not specific to SYP-1 (Figure S2).

P-granules are germline RNA storage compartments that are composed of mRNAs and proteins; these include GLH-1 and PGL-1 proteins that are important for P-granule function. A recent paper by Bilgir et al., 2013 described failure in SC assembly in glh-1 mutants. Since GLH-1 is known to be regulated by CSN-5 [55], SYP aggregation could be induced by a reduction in function of GLH-1 (and the consequent P-granule defects). CSN-5 promotes GLH-1 stabilization by competing with KGB-1 for binding to GLH-1 [51,52]. If CSN-5 influenced SC through its role in P-granule function, than glh-1 mutants should show similar phenotypes (SC aggregation) to csn-5 mutants and kgb-1 should destabilize SC (lack of SC). We did not observe any changes in SC structure, or any aggregation, in kgb-1 and glh-1 mutants (Figure S3). We conclude from this, that P-granule destabilization is likely not the cause of SYP aggregation in csn mutants.

csn-2, csn-5 and csn-6 mutants affect CR assembly

Having determined all three csn mutants display SYP aggregation, we asked if the defects in SC assembly were indistinguishable in our mutants. Not all nuclei within the transition-like zone and pachytene-like zone had aggregates. The gonads were divided into six zones sized as in Coloiaacovo 2003 ([4], Figure 2A) and were scored for the percent of nuclei with aggregates in each zone. Each zone represents a size unit (36 μm x 36 μm window) organized sequentially (zone 1 being the distal pre-meiotic tips (PMT) and zone 6 the proximal late pachytene region). This division into zones was performed according to the standard protocol for quantitative analysis of early to mid-meiotic events in the C. elegans germline, (e.g., RAD-51 analysis, also see Materials and Methods). We divided the SYP-1 localization pattern to 6 categories and quantified the percent of nuclei in each category in each zone. Linear refers to SYP-1 that is in linear function of GLH-1 (and the consequent P-granule defects). CSN-5 promotes GLH-1 stabilization by competing with KGB-1 for binding to GLH-1 [51,52]. If CSN-5 influenced SC through its role in P-granule function, than glh-1 mutants should show similar phenotypes (SC aggregation) to csn-5 mutants and kgb-1 should destabilize SC (lack of SC). We did not observe any changes in SC structure, or any aggregation, in kgb-1 and glh-1 mutants (Figure S3). We conclude from this, that P-granule destabilization is likely not the cause of SYP aggregation in csn mutants.

SYP localization in the csn-2; csn-5 double mutant revealed similar findings; however early meiotic nuclei tended to have low SYP-1 localization in the csn-2 double mutant (Figure 2B), while later meiotic nuclei were more similar to csn-5 (Figure S4). Overall, the percent of aggregated nuclei varied between gonads (e.g., 50–72% for csn-5), but mutant gonads always contained SYP-1 aggregates and wild-type gonads never harbored SYP-1 aggregates. The early appearance of SYP aggregates as SC assembly initiates (zone 2–3) indicates that the primary defect observed in csn mutants is in SC assembly.

SYP-1 aggregation could result from over-expressing SYPs [12]. To address this point; we performed a Western blot analysis to determine the level of SYP-1 in the csn-2 and csn-5 mutants. Both
**csn** mutants had a reduced level of SYP-1 compared to wild-type (Figure 2G, csn-2 40% ± 23 and csn-5 80% ± 5 of wild-type, average between experiments). We also performed a similar experiment using HTP-3 as a normalization control and found similar results (Figure S5 csn-2 76% ± 15 and csn-5 65% ± 6 of wild-type). We also utilized a cytology-based assay to quantify the amount of nuclear SYP-1 protein in csn nuclei compared to wild-type. In this analysis, the intensity of an image was used for calculating the amount of protein using standard methodologies (for details see Materials and Methods). We observed a decrease in nuclear SYP-1 in the csn-5 mutant, but not for csn-2, compared to wild-type (Figure S5). These data argue that the SYP-1 aggregates are not the result of detectable over-expression of SYP-1.

**csn-2 and csn-5 are required for gonad proliferation and fertility**

The overall length of the gonads of csn mutants is shorter than observed in wild-type (Table S4), which could result from reduced proliferation of mitotic cells. If mitotic proliferation (prior to meiotic entry) was affected, the size of the mitotic zone (i.e., the PMT) would be shorter in csn mutants. As nuclei enter meiosis, they acquire a polarized configuration of chromosomes indicating meiosis was initiated. We used this polarization to measure the length of the PMT of gonads for each genotype. Our analysis focused on csn-2 and csn-5, although csn-6 showed similar phenotypes that were not quantified in such detail. In the csn mutants, the PMT region was 60% and 77% of the length in csn-2 and csn-5 respectively.
Figure 2. Quantification of the SYP-1 aggregates. A) Schematic representation of the zones of the C. elegans gonad. PMT = pre-meiotic tip, TZ = transition zone, EP = early pachytene, MP = mid pachytene, LP = late pachytene. B–E) Quantification of SYP-1 aggregates in zones of the gonad. Percent of nuclei with: no SYP-1 (black), linear SYP-1 (blue), aggregated SYP-1 (purple pink and red) and other (yellow), zones as in A, n nuclei scored wild type: 1123, csn-2: 868, csn-5: 1020, csn-6: 85, p < 0.0005 for pairwise comparisons; Fisher’s Exact Test F) Representative images of nuclei scored in C–D all taken from the same gonad in late pachytene of csn-2 mutants, G) Western Blot confirming the reduction of expression of SYP-1 in csn mutants. Normalization values (α-SYP-1/α-TUB) shown are the average of 2 different experiments. Normalized intensities: wild-type 0.72 ± 0.26, csn-2 0.24 ± 0.20 and csn-5 0.58 ± 0.11.
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Similar analysis using HTP-3 antibody as a marker for meiotic entry revealed similar findings: PMT region was ~60% shorter than in wild-type for both mutants (for each strain p = 0.004 for wild-type vs. csn-2; p = 0.002 for wild-type vs. csn-5 and p < 0.05 for csn-2 vs. csn-5; Mann Whitney Test). One interpretation of these data is that the transition from mitosis to meiosis occurs earlier in these mutants compared to wild-type.

When nuclei move to diakinesis, the final stage of meiotic prophase I, wild-type gonads contain an average of 8.1 ± 1.1 diakinesis nuclei. These diakinesis nuclei are also referred to as oocytes [56], although the cellularization process occurs only towards the end of diakinesis. Unlike wild-type, most gonads of csn homzygotes lacked diakinesis nuclei/oocytes (Figure 3B). In csn-2 mutants, only about 25% of the gonads had diakinesis nuclei, and an average of 0.8 ± 0.76 per gonad (Figure 3C). In csn-5 mutants, 65% of the gonads had diakinesis nuclei with an average of 1.23 ± 0.98 per gonad. We performed an egg lay assay to determine the number of eggs laid and their viability. For the csn mutants, no eggs were laid in a three-day period; in contrast, wild-type worms had an average of 247 ± 16 eggs laid per worm (Figure 3C).

csn-2, csn-5 and csn-6 are required for pairing stabilization

Pairing interactions between homologous chromosomes are initiated in a SC-independent manner at specific chromosomal...
sites (pairing centers). The term pairing stabilization describes pairing interactions that spread outside the pairing centers and lead to the persistence of homolog association throughout pachytene. In syp mutants, loci distant from the pairing centers exhibit a very low level of pairing throughout meiosis [54]. Since the data indicated that csn mutants lack a fully functional SC, we expected to find that pairing stabilization had been compromised in the csn mutants, similar to syp mutants. To test this, we used a 5S ribosomal RNA locus on the center of chromosome V to analyze pairing interactions between homologous chromosomes by fluorescence in situ hybridization (FISH). The gonads were divided into six zones and were scored for the percent of nuclei with paired 5S loci in each zone ([57], Figure 4 and Tables S1 and S2 for n values and statistics).

In zone 1, as expected, wild-type and syp-1(me17) control nuclei, as well as the csn mutants, showed little to no homologous pairing, fewer than 15% of 5S loci had paired chromosomes. As the nuclei progressed through meiotic prophase I, wild-type chromosomes initiated pairing and maintained high levels of pairing through the pachytene zones. In syp-1(me17) because there is no SC formed, mutant chromosomes remained mostly unpaired throughout the germline (Figures 4A and 4B). Pairing levels in csn-2 mutants never exceeded 20% of the 5S loci paired in any zone, indicating the majority of the chromosomes were unpaired. Overall, csn-2 and syp-1(me17) mutants exhibited similar pairing defects throughout meiotic prophase I (Figure 4A and 4B, Table S1 for n values and S2 for statistics). In contrast to csn-2 mutants, csn-5 mutants initiated pairing similarly to wild-type. Since the transition from mitosis to meiosis is occurring earlier in csn-5 mutants, pairing initiated at zone 2, compared to zone 3 in wild-type (Figure 4A). In zone 4, csn-5 mutants showed a reduction in the percent of nuclei with paired chromosomes, but the levels were intermediate between those observed in wild-type and syp-1 or csn-2 mutants. The percent of nuclei with paired chromosomes for the csn-5 mutant remained higher than the csn-2 mutant for zones 5 and 6. csn-6 showed an intermediate phenotype with low pairing levels as meiosis initiated (similar to csn-2) that then increased (similarly to csn-5), but never exceeded wild-type pairing levels. Meiotic nuclei of csn-2; csn-5 double mutants tended to have low pairing levels overall; the pairing levels in late pachytene nuclei were similar to csn-5 and statistically different from csn-2. When taken together these findings are consistent with a view where defects in SC assembly perturb pairing stabilization. The percent of nuclei with linear SYP-1 (Figure 2B-E) frequently exceeded the percent of nuclei paired. Therefore these data suggest that SYP assembled in a linear manner on chromosomes in csn mutants cannot support pairing stabilization. This SC-like localization (linear SYP-1) likely represents non-homologous synapsis and/or SYP assembly between sisters.

Meiotic recombination and crossover formation are perturbed in csn mutants

In mutants that do not form the SC, events downstream of pairing and SC assembly such as meiotic recombination are perturbed [54]. We expected the csn mutations would have a similar effect on recombination. RAD-51 is a strand exchange protein used as an indirect marker for DSB formation and subsequent repair in C. elegans [4]. The gonads were divided into zones as previously described and the numbers of RAD-51 foci per nucleus were counted (Figure 5A and Table S3 for n values and statistics).

Mitotic nuclei in zones 1 and 2 showed very low levels of RAD-51 in wild-type and csn-2 mutants. csn-5 and csn-6 mutants exhibited slightly increased levels of RAD-51 foci in mitotic nuclei. RAD-51 foci levels increased at the entrance to meiosis in all genotypes tested, as expected from the induction of meiotic DSBs. The increase in RAD-51 foci/nucleus occurred earlier in csn mutants, likely due to the fact meiotic entry occurred earlier.

In C. elegans, an obligatory crossover is observed per chromosome pair [4]. COSA-1, a conserved cyclin related protein, localizes to crossovers and can be used to monitor the number of designated crossovers per nucleus (normally six, one for each wild-type bivalent formed) (Figure 5C and D). A reduction in COSA-1 foci suggests a defect in crossover formation. We tested the csn mutants using a GFP-tagged COSA-1 [58] to determine if crossover formation was affected. COSA-1 foci were measured at the last zone of late pachytene (as in [58]). Wild-type nuclei had an average of 5.8±0.04 COSA-1 foci (10% of nuclei with less than 6 foci), indicating designated crossovers had been properly formed (Figure 5C, see legend for statistics and n values). However, in the csn-2 mutant, 95% of the nuclei had less than six foci (1±0.16 foci per nucleus). In the csn-5 mutant, we observed a wider distribution of the number of COSA-1 foci observed, with 82% of the nuclei with less than six foci (2.8±0.21 foci per nucleus). In the csn-6 mutant, we observed similar distribution to that of csn-5, with 67% of the nuclei with less than six foci (an average of 3.4±0.23 foci per nucleus). The average numbers of COSA-1 foci were significantly different between the csn-2 and csn-5 or csn-6 mutants. These data suggest a role for CSN/COP9 in crossover formation.

Apoptosis is increased in csn-2 and csn-5 mutants and dpMKP-1 levels are reduced

In synopsis-defective mutants lack of synapsis [59], as well as an accumulation of DSBs [4] results in increased apoptosis at late pachytene [54]. CED-1, is expressed during the process of engulfment; a mechanism of clearing apoptotic corpses from the germline in late pachytene. Thus, the fusion protein cep-1::GFP, exclusively surrounds apoptotic nuclei and is used as a marker to detect apoptosis.

Both csn-2 and csn-5 mutants had lower average numbers of nuclei in late pachytene (Figure 6A wild-type average = 52.2; csn-2 = 26.7; and csn-5 = 30.9). However, only csn-2 had a significant increase in apoptosis (4-fold) while csn-5 had apoptotic levels similar to wild-type (Figure 6A wild-type average = 2.96; csn-2 = 8.2; and csn-5 = 3.3 apoptosis levels were not examined in csn-6 mutants). When normalized for the number of nuclei in late pachytene, both mutants showed increased apoptosis, and, as expected, csn-2 mutants showed a larger increase. This is the more appropriate analysis since csn mutants have less germline nuclei.

There are two apoptotic checkpoints in C. elegans meiosis that are activated by unsynapsed chromosomes: the synopsis checkpoint mediated by PCH-2 [59] and the meiotic recombination checkpoint mediated by CEP-1/p53 [60]. We investigated whether removing these two genes could bypass the DNA damage/synapsis checkpoint leading to apoptosis in the csn mutants. pch-2(tm1458); csn-2(tm2823) and pch-2(tm1458); csn-5(ok1064) double mutants were generated and cep-1(RNAi) was
Figure 4. Pairing stabilization is affected in csn mutants. A) Analysis of pairing stabilization between wild-type, syp-1(me17), csn mutants. A schematic representation of the timing of meiotic stages relative to the zones in the C. elegans gonad. zone 1 = pre-meiotic tip, zone 2 and 3 = transition from mitosis to meiosis, zone 4–6 = pachytene. The black arrow represents the movement of nuclei through the stages (zones) of meiosis. csn mutants show defects in pairing stabilization number of nuclei counted and p-values can be found in Sup.Tables 1 and 2. B) High magnification micrographs of individual nuclei. Images are projections through three-dimensional data stacks. 5S FISH probe foci are in green and DAPI stained chromosomes are in blue. zone 4 = early pachytene, zone 5 = mid pachytene, zone 6 = late pachytene. Scale bar is 2 μm.

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Figure 5. Accumulation of recombination intermediates and reduced crossover formation in csn mutants. A) Analysis of RAD-51 foci in wild-type compared to csn mutants. Position along the x-axis refers to the zone in the gonad (Figure 4). RAD-51 foci accumulate upon entrance to meiosis in csn mutants. Numbers of nuclei counted and p-values can be found in Sup. Table 3. Schematic representation of the timing of meiotic stages relative to zones scored in the csn mutants compared to wild-type. B) Quantitative analysis of COSA-1 foci in late pachytene of the wild-type and zone 6-like section of the csn mutants color code for number on COSA-1 is at right. Number of designated crossovers marked by COSA-1 is reduced. Number of nuclei scored: wild-type n = 123, csn-2 n = 111, csn-5 n = 94, csn-6 n = 78 p < 0.0005 for comparison between wild-type and mutants and csn-2 to csn-5 or csn-6, p = 0.13 for csn-5 to csn-6, Mann Whitney Test. C) Micrograph images of COSA-1 foci (green), chromosomes DAPI (blue), and SYP-1 (red) in wild-type and csn mutants. D) Micrograph images of RAD-51 foci (red), COSA-1 foci (green), and chromosomes DAPI (blue) in wild-type and csn mutants. Images are projections through three-dimensional data stacks. Scale bar is 2 μm.

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Figure 6. Apoptosis and MPK-1 expression are altered in csn mutants. A) Quantification of the number of nuclei with CED-1::GFP present in late pachytene of the C. elegans gonad. Red bars represent the total number of apoptotic nuclei in the late pachytene region. The blue bars represent the total number of nuclei in the late pachytene region. Apoptosis is increased in csn-2 mutants, but not in csn-5 mutants, \( *_{pMW} < 0.0005 \). There is also a reduction of overall nuclei in the late pachytene region of the gonad in both csn mutants, \( **_{pMW} < 0.0005 \). Wild-type \( n = 25 \) gonads; csn-2 \( n = 19 \); and csn-5, \( n = 18 \), B) Analysis of SYP-1 aggregate phenotype in csn mutants and apoptosis checkpoint double mutants. Bypassing the apoptotic checkpoints reduces the number of nuclei with aggregates. Total number of nuclei counted and \( p \)-values can be found in Sup. Table 5. C)
performed on the $c sn$ mutants. Overall gonad length, number of oocytes in diakinesis, and the number of nuclei containing aggregates were measured. If increased apoptosis in late pachytene was the reason for the severe reductions in oocyte numbers, in $c sn$ mutants, then bypassing the checkpoint function should increase the numbers of diakinesis nuclei and increase the overall size of the gonad. We observed no change in overall gonad length (Table S4) between the single $c sn$ mutants and the corresponding double mutants, nor any increase in oocytes in diakinesis in young adults (one day post-L4, Figure 3C). Since clearing apoptotic corpses may be slow, we scored the same genotypes two days later, giving the opportunity for accumulation of cells (in double mutants) otherwise destined for apoptosis ($c sn$ single mutants). In $c sn$-5 mutants overall gonad length decreased with age, possibly due to the defects in mitotic proliferation (less nuclei there are, the gonad gets shorter). However, both $c sn$-5; $p c h$-2 and $c sn$-5; $c e p$-1 mutants showed increased gonadal size compared to $c sn$-5 single mutants (almost double the size, table S4). These data indicate that both the synopsis and the DNA damage checkpoint are activated in $c sn$-5 mutants and are clearing nuclei through apoptosis.

Next, we assayed how removing the DNA damage and synopsis checkpoints would affect SC morphology in $c sn$ mutants. For this analysis we scored two categories: nuclei with linear SYP-1 localization and nuclei with aggregates (with/without other analyses we scored two categories: nuclei with linear SYP-1 and nuclei with aggregates (with our without other forms of SC). We measured the percent of nuclei with aggregates in double mutants with perturbed apoptotic machinery compared to single mutants. $c sn$-5(ok1064); $p c h$-2(tm1458), $c sn$-2(tm2823); $c e p$-1(RNAi), and $c sn$-5(ok1064); $c e p$-1(RNAi) double mutants exhibited a decrease (2-fold) in the number of nuclei with SYP-1 aggregates in late pachytene compared to the respective single $c sn$ mutant (Figure 6B and Table S5 for statistics). Therefore, nuclei with linear SYP were more frequently found in double mutants (in contrast, 82% of wild-type and 75% of $c sn$-2 mutants had dpMPK-1 staining in late pachytene while only 3% of the $c sn$-5 mutants had dpMPK-1 staining. In contrast, 82% of wild-type and 75% of $s y p$-1(me17) gonads had dpMPK-1 staining (Figure 6C). $M P K$-1 has two isoforms in $C$. elegans, $M P K$-1A (43.1 kD) which is mostly somatic and $M P K$-1B (50.6 kD) is germline specific [60,63]. We quantified the intensity of the bands and normalized to the tubulin controls (Figure 6D). In wild-type, both isoforms were detected with $M P K$-1A having an average normalized intensity of 2.27. The $M P K$-1A band was detected in both $c sn$ mutants ($c sn$-2 = 0.99 and $c sn$-5 = 0.96 normalized intensities), although it was 2-fold lower in both mutants. The germline $M P K$-1B had an intensity of 9.91, but was not detected in either $c sn$ mutant (Figure 6D). These data indicate that $c sn$ mutants lead to reduced MAPK/$M P K$-1 signaling which almost completely blocks pachytene exit and severely reduces oocyte numbers. This data is consistent with the observation that removing apoptosis checkpoints ($p c h$-2 or $c e p$-1) could not increase oocyte numbers in $c sn$ mutants: even if more nuclei survived apoptosis, they could still not exit pachytene arrest in the absence of dpMPK-1.

Decreasing neddylation and ubiquitination modify the phenotypes observed in $c sn$ mutants

We have shown that synopsis (SC assembly) and recombination are perturbed in three $c sn$ mutants. The main role of the CSN/COP9 signalosome is in deneddylation of CRLs. Therefore, the $c sn$ mutant phenotypes could be attributed to the increased neddylation in the absence of a functional CSN/COP9 signalosome. The effect of complete absence of neddylation on the germline cannot be examined since null mutants in ned-8, the only gene encoding for the small modifying protein NED-8, are larval lethal. To test the hypothesis that over neddylation leads to the phenotypes described we have decreased the levels of neddylation via RNAi for ned-8 in $c sn$-2 and $c sn$-5 mutants. As seen in Figure 7B and D, (detailed distribution in Figure S6 and statistics in Table S6), ned-8(RNAi) partially suppressed the synopsis defects of $c sn$ mutants ($p L 4 4 4 0 = empty vector control vs. ned-8(RNAi) on $c sn$-2 $p < 0.001$, on $c sn$-5 $p = 0.002$, Fisher’s Exact Test). More strikingly the levels of designated crossover (COSA-1) of $c sn$ mutants were partially restored (Figure 7E). Since CSN/COP9 signalosomes typically deneddylates CRLs, which are ubiquitin ligases, the $c sn$ mutant phenotypes could also potentially be suppressed by reducing ubiquitination levels. As with neddylation, null mutants in genes essential for the ubiquitination pathway die prior to the adult stage and cannot be used in our studies. We have used RNAi for the sole E1 ubiquitin ligase of $C$. elegans, UBA-1, to test the hypothesis that the phenotypes of $c sn$ mutants can be attributed to increased ubiquitination. As with neddylation, uba-1(RNAi) partially suppressed both the defects in SC assembly (Figure 7 B and D) and the crossover defects of $c sn$ mutants (Figure 7E). If imbalance of neddylation is the cause of the phenotype observed in $c sn$ mutants, hyper-neddylation ($c sn$ mutants) and hypo-neddylation (mutants in the NED-8 pathway) will lead to similar phenotypes. The ned-8(RNAi) is weak; it does not lead to increased lethality although the null allele has a lethal phenotype. Therefore, it is not surprising that ned-8(RNAi) on wild-type did not lead to any phenotype. However, examination of an E1 NED-8 ligase (zfl-1) revealed defects in SC assembly reminiscent of the $c sn$ mutant phenotype (Figure 7G), defects not observed in the control (Figure 7F). Finally, we sought to identify the E3 CULLIN ligase, which is the target of CSN/COP9 signalosome. This analysis is limited because many mutants of cul genes are embryonic or larval lethal. We have identified one cul-4 mutant allele (a C-terminal truncation) that exhibited mild defects in SC assembly, including aggregation of SYP-1 (Figure 7H).
CUL-4 is therefore, a possible CSN/COP9 signalosome target. This genetic analysis is consistent with a canonical role for CSN/COP9 signalosome in the CRL pathway: regulating CUL-4 via denaddylation and keeping the balance between neddylation and denaddylation.

Taken together, these data indicate the CSN/COP9 signalosome has multiple roles in meiosis: the signalosome affects the number of germline nuclei, SC assembly and stabilization, recombination, MAPK signaling and promoting pachytene exit.

Discussion

CSN/COP9 is required for chromosome synapsis, pairing and recombination during C. elegans meiosis

The CSN/COP9 signalosome has diverse and well-documented somatic functions, yet the understanding of its role in meiosis is limited [28]. Studies of the C. elegans and D. melanogaster CSN/COP9 indicate it plays a critical role in the regulation of Vasa/P-granule proteins in the germline [42,55,67]. Here, we show CSN/COP9 has a previously unknown meiotic function: it is essential for proper SC assembly, independent of its P-granule role in the germline. We demonstrate that events following SC assembly (e.g., stabilization of homolog pairing interactions and the repair of meiotic DSBs) are perturbed as well. The three csn mutants show similar, but not identical effects on these processes; in the absence of CSN/COP9, the SYPs (CR proteins) aggregate. In C. elegans, stabilization of pairing interactions is absolutely dependent on SC formation and independent of DSB formation and repair [57]. Therefore, it is reasonable to propose that the pairing defects observed in CSN/COP9 mutants stem from defects in SC formation. The limited amount of SC that assembles on chromosomes in csn mutants cannot support wild-type levels of stabilization of pairing interactions (by FISH analysis). In the absence of fully stabilized pairing interactions, unresolved recombination intermediates (marked by RAD-51) accumulate. This leads to a reduction in the numbers of designated crossovers (marked by COSA-1 foci) in csn mutants and an elevation of apoptosis. The magnitude of these phenotypes in csn-2 mutants

Figure 7. csn mutant genetically interact with the ubiquitination-neddylation pathway in the regulation of SC assembly and recombination. A–D) Quantification of SYP-1 aggregates. A and C are data from all gonad, while B and D is from late pachytene nuclei. Percent of nuclei with: no SYP-1 (black), linear SYP-1 (blue), aggregated SYP-1 (purple pink and red) and other (yellow), zones as in Figure 2A, n nuclei scored for whole gonad csn-2; with pL4440 = 2023 with ned-8(RNAi) = 430, with uba-1(RNAi) = 1121, csn-5; with pL4440 = 2096, with ned-8(RNAi) = 441, with uba-1(RNAi) = 1014. E) Quantitative analysis of COSA-1 foci in late pachytene wild-type. Percent of nuclei with: zero (black) one (orange), two (red), three (pink) four (magenta), five (purple), six (blue) and seven (gray), n nuclei scored for pL4440 = 57, with ned-8(RNAi) = 47, with uba-1(RNAi) = 25, csn-2; with pL4440 = 168, with ned-8(RNAi) = 66, with uba-1(RNAi) = 126, csn-5; with pL4440 = 152, with ned-8(RNAi) = 47 with, uba-1(RNAi) = 83, pL4440 = empty vector control vs. ned-8(RNAi) on csn-2 or csn-5 p = 0.002, p < 0.001, Fisher’s Exact Test, pL4440 vs. uba-1(RNAi) on csn-2 or csn-5 p < 0.001 Mann Whitney Test). F–H) Quantitative analysis of SYP-1 aggregates in zones of the gonad for the indicated genotypes, as performed in figure, number of total nuclei scored: wild-type 25 n = 641, rfl-1 at 25 n = 1674, cul-4 n = 542 2, I) Schematic representation of the pathway examined in the experiment.

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closely resembles that of syp null mutants, supporting our model that the later meiotic defects (pairing and recombination) stem from an inability to form functional SC. The exact magnitude of the effects on recombination and pairing initiation is different between the mutants and may point to additional roles of components of the CSN/COP9 signalosome in pairing and recombination (more discussion below). Importantly, all three csn mutants we have examined affect synopsis, pairing, and recombination and therefore we are confident in our claim for a role for the CSN/COP9 complex in these key meiotic events.

CSN/COP9 is required for normal levels of germline proliferation, MPK-1 activation and pachyneme exit

Consistent with previous studies in C. elegans utilizing RNAi [51,52] the three csn mutants have a reduced gonad size. Our data suggest this reduction is due to a proliferation defect, as the number of mitotically dividing nuclei in the pre-meiotic tip is reduced in the csn mutants. Drosophila csn4 and csn7 mutants cannot stabilize Cyclin E leading to defects in cell cycle progression of mitotically proliferating germ line nuclei [46]. This suggests a conserved function of the CSN/COP9 signalosome in pre-meiotic germ line proliferation.

Once nuclei of csn mutants enter meiosis, chromosomes cluster to one side of the nucleus as in wild-type; unlike wild-type however, a portion of these nuclei do not re-acquire the normal dispersed chromosomal organization as they progress through meiosis (Figure 1B and C). This phenotype of persistent polarized chromosome organization is reminiscent of syp null mutants during meiotic progression. This finding, together with the observation that csn mutants do initiate meiotic recombination and form some designated crossovers, is consistent with meiotic progression from leptotene to pachyneme in these mutants. However, unlike syp mutants, csn mutants produce almost no diakinesis/oocyte nuclei. Reduced oocyte production would result from the decrease in numbers of germ cells, yet the effect on oocyte production is greater than expected from the reduction in number of pachyneme nuclei destined to be oocytes. We have found MAPK signaling (dpMPK-1) is reduced in csn mutants. MAPK signaling is essential for pachyne progression [63] and so we infer that reduced dpMPK-1 levels are likely the primary contributor to the severe reduction in oocyte numbers in csn mutants. As synopsis defective mutants (e.g., syp-1) still exit pachyneme and form oocytes in comparable levels to wild-type, the lack of MAPK signaling in the csn mutants defines another function for the CSN/COP9 complex and is not a secondary effect of the synopsis defects. Since CSN-5 physically interacts with MPK-1 [61,62], the absence of MPK-1 phosphorylation, may be due to the absence of this interaction. The role of the CSN/COP9 signalosome in pachyneme exit seems to be conserved, as similar to our observation, Drosophila csn8 and csn4 mutants arrest at the pachyneme-diploneme transition [68,69].

The relation between the linear and the aggregate forms of the CR proteins

In C. elegans, CR/SYPs assembly can be misregulated in certain meiotic mutants without forming aggregates [7,9,11,14]. These aberrant forms of SC assembly appear to be fully formed SCs that are assembled in the wrong chromosomal context. The situation found in the csn mutants is different: in addition to aberrant SC assembly (short stretches ~50% of nuclei contain one SYP aggregate. In C. elegans, lack of any one of the four SC protein results in elimination of the other SYPs without their aggregation [13], indicating that mechanisms exist to remove SYPs not bound to DNA. csn mutants are therefore likely perturbed in mechanisms designed to clear aggregated SYPs, and assemble a “SC-like” structure which is invisible to the degradation machinery. In yeast, the CR protein is continually loaded on the SC, even in mid-prophase [70]. If a similar rapid exchange of SYPs occurs during C. elegans meiosis, SC assembly defects (problems in SC assembly upon meiotic entry) and SC stabilization defects (throughout pachyneme) are related, due to continuous assembly of SC protein occurring throughout prophase. This may explain why a mutant that affects SC assembly (csn-5) was retrieved in an enhancer screen for a mutant showing SC disassembly defects.

Although it is likely that the formation of CR aggregates reflects an aberrant form of SC assembly, it is not clear if these aggregates are the problem or an attempt to solve a problem. In other words, it could be that the CRs which cannot be properly loaded onto chromosomes aggregates and therefore would not be available for SC formation. Alternatively, CRs may be loaded on to chromosomes, but then identified as aberrant CR, removed and then form aggregates which acts as reservoirs of CR proteins waiting to be reloaded. Since linear (and non aggregated CR) seem to be a better predictor for failed meiosis, we tend to favor the hypothesis were CR aggregates are formed in response to attempts to correct an aberrantly formed SC. First, mutants with more linear SYP-1 (csn-2 and csn-6) in early meiotic stages (zone 3) show more severe defects in pairing compared with a mutant with a higher percentage of SYP-1 aggregation (csn-5). Second, nuclei with linear SYP-1 are preferentially selected for elimination by apoptosis. Lastly, if aggregation is only an assembly defect, then the percent of aggregated nuclei should increase as meiosis progresses, which does not occur. This is more consistent with a model of SYP shuffling between an assembled and an aggregated from.

We have shown that the percent of nuclei undergoing apoptosis is increased in both csn-2 and csn-5 mutants. This active elimination of nuclei by apoptosis reduces the size of the germline over time (csn-5; pch-2 gonads were longer compared to csn-5 single mutant at day 3). Both the synopsis checkpoint and the DNA damage checkpoints contribute to the elimination of nuclei in csn-5 mutants. Checkpoint activation is associated with an increase in aggregated SYP-1. However, while the percent of nuclei with aggregated SYP-1 is reduced by removing both checkpoints, the removal of the synopsis checkpoint (pch-2 mutant) affects only the csn-5 mutants. Pairing levels in csn-5 mutants are higher than observed in csn-2 mutants; it is possible that csn-5 mutants attempt more to synapse (and fail) more compared to csn-2 mutants, which leads to a robust activation of the synopsis checkpoint. PCH-2 serves as a kinetic barrier for synopsis, slowing down synopsis [71]. Therefore, it is possible that elimination of pch-2 in the csn-2 background increases the percent of linear SYP-1 merely by increasing the speed of assembly. Although this is possible, the removal of cep-1, which is not known to act like pch-2, has the same effect on the reduction of nuclei with SYP-1 aggregates. We propose that the reduction in fraction of nuclei with SYP-1 aggregation in a checkpoint mutant is due to the removal of the apoptotic program. This may be done directly by inducing changes in SC morphology or by activating downstream meiotic arrest providing more time for SC elongation. Alternatively, it could be done directly by preferentially eliminate nuclei with linear SYP-1.

How CSN/COP9 regulates chromosome synopsis

There are three known examples of PC-like structures in C. elegans mutants: csa-1; spo-11 double mutants [14], pgl-1 mutants
at 25°C and higher [20] and dynein mutants in early prophase [21]. Our analysis thus far is consistent with a different function for the CSN/COP9 signalosome; aggregates in csn mutants are found in the presence of DSBs (unlike cra-1), at normal growth temperatures (unlike pgl-1 mutants), and throughout the germine (unlike dynein mutants). Therefore, we propose that CSN/COP9 participates in SC assembly in a novel manner. The signalosome's role is not merely due to promoting SYP degradation; we confirmed using several assays that csn mutants do not show increased SYP-1 levels.

Pathways of SC assembly involve post-translational modifications of SC proteins. These modifications could facilitate CR protein association with chromosomes and prevent their aggregation. In yeast, it was shown sumoylation promotes lateral element [72] and CR [73] assembly. Mouse SC assembly is regulated by phosphorylation of lateral element proteins [74]. In C. elegans, SYP-1 and SYP-2 appear to be post-translationally modified, however, the precise identities of these modifications is unknown [13]. All four SYPs contain potential sites for phosphorylation, ubiquitination, and sumoylation. In C. elegans, an evolutionarily conserved ubiquitin/sumo modifier [75] is linked to SC disassembly, but is not required for SC assembly [76].

The discovery that the CSN/COP9 complex is required to prevent SYP aggregation raises the question of whether CSN/COP9 is involved in post-translational modification of the SYPs to prevent their aggregation. SYP aggregates contain all four SYP proteins, hence one aggregation-prone SYP may lead to the capture of all SYPs. The CSN/COP9 complex's activity in deneddylation has been well documented, but it also possesses Ser/Thr kinase and deubiquitination associated activities [28]. CULLIN RING E3 ligases (CRLs) are the most well studied of the CUL-4 to regulate SC assembly. If mutants show defects in meiotic entry and meiotic recombination, PLOS Genetics | www.plosgenetics.org 14 November 2014 | Volume 10 | Issue 11 | e1004757

CRL. This suggests that both hyper and hypo-neddylation is conserved ubiquitin/sumo modifier [75] is linked to SC disassembly [82,83]. The CRL is driving the stabilization of early prophase pairing and crossovers (5S FISH) and double the numbers of pairing and crossovers, while mutants in the other subunits have no clear meiotic phenotypes [84]. CSN5 [39,85] and CSN2 [86] are the only subunits shown to act outside CSN/COP9 in vivo.

We have shown that csn-2, csn-5 and csn-6 mutants all lead to defects in SC assembly, a reduction in pairing, and increase in DSB repair defects in meiotic recombination. However, the magnitude of these effects varies (see summary in Figure S7).

Pairing analysis show that the csn-2 mutants almost mimic a syp null mutant, while the csn-5 and csn-6 mutants show milder pairing stabilization defects (5S FISH) and double the numbers of designated crossovers (COSA-1) compared to the csn-2 mutant. The SC is driving the stabilization of early prophase pairing interactions (zone 2–3), while later events (zone 6) are also promoted by the stabilizing role of crossovers, which are likely higher in the csn-5 and csn-6 mutant (COSA-1). Therefore, as for pairing and crossover formation, csn-5 and csn-6 both display milder phenotypes compared to csn-2. However, when examining synopsis (SYP-1) and the kinetics of repair of recombination intermediates (RAD-51), csn-2 and csn-6 cluster together with milder phenotypes compared to csn-5. It is hard to reconcile this model with a strictly linear role for the CSN/COP9 signalosome affecting synopsis, pairing, recombination progression and ending at crossover formation. It is likely that the picture is more detailed and complex. We propose that in addition to the central role of the CSN/COP9 signalosome in SC formation (which affects recombination and pairing stabilization) additional roles exist in down-regulating pairing initiation and promoting crossover formation, independently of the SC. These different roles may stem from alternative complex formation, as was shown in other organisms. One possible model would involve an inhibitory role for CSN-5-CSN-6 on pairing stabilization outside the CSN/COP9 holocomplex. CSN-5 and CSN-6 have been shown to physically interact and form a sub module in the CSN/COP9 complex [78]. We do have some support for a role for CSN-5 in down-regulating pairing initiation: as expected by this model, csn-5 is epistatic to csn-2 as for pairing interactions.

Identifying the precise role of each subunit and sub-complexes in meiosis will require more extensive analysis of each subunit. The work presented here is but a first step in this direction. It is intriguing that both neddylation and deneddylation is key to accurate meiosis and these processes are likely to be evolutionarily conserved.

**Materials and Methods**

### Strains

Most *C. elegans* strains were cultured under standard conditions at 20°C [87]. Several strains (in bold) were maintained at 15°C and experimentally cultured at 26°C. N2 Bristol worms were utilized as the wild-type background. The following mutations and chromosomal rearrangements were used:

- **LG I:** cep-1 (ep547), csn-2 (tm2823), glh-1 (gh100), hT2 [bli-4(e937) qIs48]
- **LG II:** ceh-1 (tm1458), cul-4 (ok1891)
- **LG III:** rgl-1 (or198)
- **LG IV:** csn-5 (ok1064), csn-6 (ok1604), hkb-1 (um3), nT1 [qIs50]
- **LG V:** syp-1 (me17)

The following transgenic lines were used: *meh8* (GFP::COSA-1), sm634 [ced-1p::ced-1::GFP+rol-6 (su10060)] and *mek9* [unc-19(+); pep-1 promoter: gfp::SYP-3]; *unc-119(ei3) III* [88]. All strains were outcrossed 6 times except gh1-1 (gh100) which was

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### CSN subunits acting outside the CSN/COP9 complex

The CSN/COP9 complex is composed of 7 to 8 subunits, depending on the organism [28]. The CSN5 subunit is responsible for the deneddylation activity of the CSN/COP9 complex, but cannot function in deneddylation outside the holoenzyme [77,78]. Smaller sub-complexes, with variable subunit composition have been isolated as well: CSN4-7 and CSN-4-5-6-8 in mammals [81]. Mutant analyses have indicated the loss of any one subunit leads to signalosome phenotypes [84]. CSN5 [39,85] and CSN2 [86] are the only subunits shown to act outside CSN/COP9 in vivo.
outcrossed twice, pch-2(tm1458) outcrossed 5 times and cul-4(ok1891) which was outcrossed once.

Analyses of the csn allele transcripts by RT-PCR

In order to determine if the deletions in the csn mutants are in-frame or out of frame, we conducted RT-PCR analyses. This was done using the Superscript III OneStep RT PCR kit (12575-026, Life Technologies) and the primers TGAATACGAAGATGAGATAGTTGGGC1 and CAATACGCCCTCTGACCAAAAGA for csn-2 and CGAAGGTCTTTGCGATCTCTGG and GCA-GATTTGCTTTGGAAGCTTG for csn-5. Our analysis reveals that csn-2(tm2823) is an in-frame deletion. The csn-2(tm2823) transcript lacks exon 2, and results in a 139 amino acid deletion of the peptide sequence. Although this deletion is in-frame, 28% of the protein is missing, including half of the PAL domain. We did not assess whether the total levels of transcript in this mutant are reduced. csn-5(ok1064) is a deletion that includes the promoter region and half of the coding region. Translation from the first in-frame AUG will lead to 75 amino acid peptide, a deletion of 80% of the protein, including the catalytic MPN domain. We have not yet succeeded in amplifying a csn-5 transcript form csn-5(ok1064), suggesting that csn-5(ok1064) lacks a functional promoter. The csn-6(ok1604) is in frame deletion that includes deletion of half of the gene. The csn-6(ok1604) transcript is split from the middle of exon 1 to the start of exon 3, when exon 2 is skipped, resulting in deletion of coding sequence expected to lead to 193 amino acid deletion of the peptide sequence. Therefore, 45% of the protein is missing, including most of the MPN domain. We did not assess whether the total levels of transcript in this mutant are reduced.

Immunostaining and microscopy

Adult hermaphrodites 20 h post-L4 were dissected to release gonads. DAPI and immunostaining was performed as described in [4]. For transgenic lines utilizing GFP fusions, fixation was in methanol for 1–5 minutes, then washed and prepared for microscopy as in [4]. Whole mount worms were prepared by Carney’s Fixation. Antibodies were used at the following dilutions: α-SYP-1, 1:500; α-SYP-4, 1:500; α-HIM-3, 1:500; α-HTP-3, 1:500; α-RAD-51 1:10,000; α-dpMPK-1 1:500 (Sigma). The secondary antibodies used were: Alexa Fluor 488 α-mouse, Alexa Fluor 488 α-rabbit Alexa Fluor 555 α-rabbit, Alexa Fluor 568 α-goat, Alexa Fluor 568 α-quinea pig (Invitrogen), and DyLight 594 α-goat (Jackson Immunochemicals, West Grove, PA).

The images were acquired using the DeltaVision wide-field fluorescence microscope system (Applied Precision) with Olympus 100×/1.40- or 60× numerical aperture lenses. Optical sections were collected at 0.20-μm increments with a coolSNAPHQ camera (Photometrics) and deconvolved with softWoRx software (Applied Precision). Gonadal and nuclei images are projections halfway through three-dimensional data stacks (Multiple 0.2-μm slices/stack), except of where full projections are indicated, and were prepared using softWoRx Explorer 1.3.0 software (Applied Precision) or FIJI [89].

Aggregates were defined as SYP signals with width larger than that of wild-type SC. When measured, even the smallest aggregates were larger than the larger SC width measured and above the average SC with plus 2 standard deviations. As indicated in the results section, these values were highly statistically significant (p < 0.001), indicating that our calling of SC aggregates was precise.

Quantitative analysis of the intensity of SYP-1 signals was performed on images taken from the same slide in the same exposure time. Images were analyzed using ImageJ [89]. This was performed under guided model option with a freehand polygon section in all Z-stacks of a particular SYP signal and to multiple gonads from each genetic background. We set a threshold of 250 for the max grey value being measured, to ensure that overexposed images were not included in the analysis. Data for each nucleus is the sum of all the Z-stakes in which the nuclei is detected on the DAPI channel. To obtain SYP-1 signal intensity, we subtracted the integrated intensity of the background of the same image from the SYP-1 integrated intensity to get the normalized integrated density [Nuclear (IntDen/Area) – Background (IntDen/Area)]. We presented the data as total integrated intensity ([Nuclear [IntDen/Area] – Background [IntDen/Area]) × Area of each nucleus] and also as IntDen/Area. The total integrated intensity is an indication of the total SYP-1 signal in each nucleus; the IntDen/Area is the average intensity of the SYP single in each nucleus. Statistical comparisons between genotypes were performed using the two-tailed Mann–Whitney test, 95% confidence interval.

Fecundity assay

To determine the fecundity of the csn mutants, single L4 worms were placed on seeded NGM plates and allowed to lay eggs for a 15 hr period. These worms were then moved to a fresh NGM plate and again allowed to lay eggs. This was repeated for a three day period. Eggs were counted for each genotype examined.

FISH and time-course analysis of chromosome pairing

The 5S FISH probe was generated as in [57] from a PCR fragment generated by amplifying C. elegans genomic DNA with the 5′-TACTTTGATCGGAGACGCCG-3′ and 5′-CTAACT-GGACTCAACGTTGC-3′ primers. Fragments were labeled with fluorescein-12-dCTP (PerkinElmer, Waltham, MA). Homologous pairing was monitored quantitatively as in [57]. The total number of nuclei scored per zone (n) from three gonads each for wild-type, csn-2 and csn-5 mutants. Statistical comparisons between genotypes were performed using the Fishers Exact Test, 95% confidence interval.

Time-course analysis for RAD-51 foci

Quantification of RAD-51 foci was performed for all seven zones composing the premeiotic tip to late pachytene regions of the germline as in Colaiacovo et al. (2003). The total number of nuclei (n) were scored per zone from three gonads each for wild-type, csn-2 and csn-5 mutants. Statistical comparisons between genotypes were performed using the two-tailed Mann-Whitney test, 95% confidence interval.

Time-course analysis for COSA-1 foci

Quantification of GFP::COSA-1 was carried out as in [58] with zone 6 selected to be analyzed. The total number of nuclei (n) were scored in zone 6 for 5 gonads. Statistical comparisons between genotypes were performed using the two-tailed Mann-Whitney test, 95% confidence interval.

Apoptosis

The csn mutants were introgressed to ced-1::GFP strain [smIs34 [ced-1p::ced-1::GFP+rol-6(+)] to assess apoptotic levels as per [90]. Images were taken at 60x and nuclei which displayed CED-1::GFP localization were counted as well as the total number of nuclei in the bend region (late pachytene). 10 different gonads were quantified. Statistical comparisons between genotypes were performed using the two-tailed Mann-Whitney test, 95% confidence interval.
Western blotting

L4 homozygote larvae were picked and aged to adults. The mouse α-dpMPK-1 (Sigma) was used as a primary antibody (1:1000). Mouse α-tubulin (1:1000; DSHB) was used as a loading control. Secondary antibodies were used as mouse antibody conjugated to horseradish peroxidase (HRP; 1:10,000). PBST-5% milk was used for incubation and blocking. Quantification was done on FIJI [89].

RNAi screen

csn-5 (BO547.1) was identified in a RNAi screen on the akir-1 background for mutants affecting SC morphogenesis. Synchronized L1 larvae were placed on NGM+AMP+isopropyl-B-D-1-thiogalactopyranoside (IPTG) plates seeded with RNAi bacterial clones from the Ahringer C. elegans RNAi library [91] or pl4440 empty vector (control). Embryonic lethality was scored visually and clones exhibiting increased lethality as compared to controls were selected for replication and further analysis. We then conducted a fecundity study to determine if the reduction in live progeny was due to meiotic or developmental defects. Clones demonstrating a reduction in the number of eggs laid were selected for cytological screening.

RNAi feeding protocols

RNAi clones are ground 6 hours–overnight in LB+ampicillin (50 ug/ml). Cultures are then seeded onto IPTG plates (see above) and left to grow overnight [91]. Either synchronized L1 or L4 larvae were placed on the plates, left to develop to adults. These adults were subjected to cytological analyses or their F1 progeny scored for viability.

Supporting Information

Figure S1 SC lateral elements do not aggregate in csn mutants. A) Micrographs of HTP-3 (red) and DAPI (blue) stained wild-type, csn-2(tm2823) and csn-5(ok1064) nuclei representing the various stages of the C. elegans gonad. Images are projections through three-dimensional data stacks. Scale bar is 2 μm. PMT = pre-meiotic tip, TZ = transition zone, EP = early pachytene, MP = mid pachytene, LP = late pachytene. B) Micrographs of HIM-3 (red) and DAPI (blue) stained wild-type, csn-2(tm2823) and csn-5(ok1064). Both HIM-3 and HTP-3 localization is not affected in the csn mutants. (TIF)

Figure S2 SYP-4 aggregates in csn mutants. A–E’) Micrographs of SYP-4 (green) and DAPI (blue) stained wild-type, csn-5(ok1064) nuclei representing the various stages of the C. elegans gonad. Images are projections through three-dimensional data stacks. PMT = pre-meiotic tip, TZ = transition zone, EP = early pachytene, MP = mid pachytene, LP = late pachytene. Agregation affects all SYP-4 and likely all SYPs. F–G*) mid pachytene nuclei of wild-type, csn-2 and csn-5 mutants, all with transgenic GFP:SYP-3 (green or gray scale) and DAPI (blue). Scale bar is 2 μm. (TIF)

Figure S3 P-granule component kgb-1 does not have SYP-1 aggregation phenotype. A–F) Micrographs of SYP-1(red, grey scale) and DAPI(blue) stained wild-type (A–C),kgb-1(um3) (A’–F’), and glh-1(1gk100) (A’–F’) mutant nuclei representing the various stages of the C. elegans gonad. Images are projections through three-dimensional data stacks. Scale bar is 2 μm. EP = early pachytene, MP = mid pachytene, LP = late pachytene. kgb-1(um3) and glh-1(1gk100) are temperature sensitive alleles. Worms cultured at 26°C do not exhibit SYP-1 aggregation. P-granules do not appear to be involved in the aggregation phenotype. (TIF)

Figure S4 SYP-1 localization in csn-2; csn-5 double mutants. Quantification of SYP-1 aggregates data from the entire gonad. Percent of nuclei with: no SYP-1 (black), linear SYP-1 (blue), aggregated SYP-1 [purple pink and red] and other (yellow), zones as in Figure 2A. n = 629. (TIF)

Figure S5 SYP-1 is not overexpressed in csn mutants. A–D) Quantification of the amount of SYP-1 in the nuclei measured from IF images (see Materials and Methods). Reduced nuclear SYP-1 localization in csn-5 mutants, while no effect is observed in csn-2. E) Western analysis confirming the reduction of expression of SYP-1 in csn mutants. Normalization values (α-SYP-1/α-HTP-3) shown values are of the experiment presented. (TIF)

Figure S6 SYP-1 localization in response to uba-1(RNAi) and ned-8(RNAi). SYP-1 localization throughout the germline of the indicated genotypes: A–C) csn-2 mutants D–F) csn-5 mutants, G–I) wild-type. Percent of nuclei with: no SYP-1 (black), linear SYP-1 (blue), aggregated SYP-1 (purple pink and red) and other (yellow), zones as in Figure 2A. Note that this RNAi was performed not to full penetrance to allow analysis of the germline (allow recovery of adults), n nuclei scored for whole gonad wild-type: with pl4440 = 1003, with ned-8(RNAi) = 653, with uba-1(RNAi) = 812. csn-2: with pl4440 = 2023 with ned-8(RNAi) = 430, with uba-1(RNAi) = 1121, csn-5: with pl4440 = 2096, with ned-8(RNAi) = 441, with uba-1(RNAi) = 1014. (TIF)

Figure S7 Schematics of the phenotypes observed in csn mutants. A) Comparison between each csn allele and wild-type for the indicated phenotypes on the left, B) Comparison between each csn-2 and csn-5 allele, csn-2, csn-5 double mutants and wild-type for the indicated phenotypes on the left. (TIF)

Table S1 Number of nuclei counted for FISH analyses. The numbers of nuclei counted for each zone and each genotype and the total number of nuclei for the FISH experiments. (DOCX)

Table S2 p-values calculated by Fisher’s Exact Test for all pairwise comparisons of FISH data. The p-values for pairwise genotype in each zone and for the FISH experiments. (DOCX)

Table S3 p-values and total number of nuclei counted per zone for RAD-51 analyses. Top- the numbers of nuclei counted for each zone and each genotype and the total number of nuclei for the RAD-51 foci analysis. Bottom- the p-values for the pairwise genotype comparisons in each zone and for the RAD-51 foci analysis. (DOCX)

Table S4 Average gonad lengths from PMT to dipotene and p-values for pairwise comparisons between the single mutant control and the double mutants. 1 day adult (24 hours post L4) is the standard age for examining meiotic events. 3 days adults (72 hours post-L4) were also analyzed for the genotypes indicated. (DOCX)

Table S5 p-values and total number of nuclei counted in late pachytene zone for apoptotic analyses for the genotypes indicated. (DOCX)
Table S6  p-values and total number of nuclei counted for RNAi experiments for uba-1 and nrd-8 (see Figure 7) for the genotypes indicated. Analysis for SYP-3 association is done using Fisher’s Exact Test, while analysis for COSA-1 foci numbers is done using Mann Whitney U Test. SVP-1 all genotypes is all values for zones 1 through 6. % aggregates or average number of COSA-1 foci are for each pair of genotypes compared in the statistical test, by the order they appear on the top (e.g., bottom right corner: 2.8 is average number of foci for pL-4440 on csm-5 and 5.0 is for uba-1(RNAi) on csm-5). (DOCX)

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Author Contributions

Conceived and designed the experiments: SS HB. Performed the experiments: HB MD NB SS BA. Analyzed the data: HB MD NB SS BA. Contributed reagents/materials/analysis tools: SS. Wrote the paper: HB SS.

References

1. Hassold T, Hunt P (2001) To err (meiotically) is human: the genesis of human aneuploidy. Nature Reviews Genetics 2: 289–291. doi:10.1038/35066065.
2. Hirose Y, Suzuki R, Ohira T, Minohara Y, Matsuhara H, et al. (2011) Chromatid Promote Monopolar Attachment of Sister Chromatids and Their Co-Segregation toward the Proper Pole during Meiosis I. PLoS Genet 7(3): e1001329. doi:10.1371/journal.pgen.1001329.
3. Page SL, Hawley RS (2004) The genetics and molecular biology of the synaptonemal complex. Annu Rev Cell Dev Biol 20: 525–550. doi:10.1146/annurev.cellbio.20.010303.103003.
4. Colaiacovo MP, MacQueen AJ, Martinez-Perez E, McDonald K, Adamo A, et al. (2003) Synaptonemal Complex Assembly in C. elegans Is Dispensable for Loading Strand-Exchange Proteins but Critical for Proper Completion of Recombination. Developmental Cell 4: 463–474. doi:10.1016/S1534-5807(03)00325-6.
5. Zetka M, Kawasaki I, Strome S (1999) Synapsis and chiasma formation in Caenorhabditis elegans require HIM-3, a meiotic chromosome core component that functions in chromosome segregation. Genes & Development 13: 2673–2683. doi:10.1101/gad.131104.
6. Couteau F, Nabeshima K, Villeenueve A, Zetka M (2004) A Component of C. elegans Meiotic Chromosome Axes at the Interface of Homolog Alignment, Synapsis, Nuclear Reorganization, and Recombination. Current Biology 14: 545–552. doi:10.1016/j.cub.2004.03.033.
7. Couteau F (2005) HTP-1 coordinates synaptonemal complex assembly with homolog alignment during meiosis in C. elegans. Genes & Development 19: 2744–2756. doi:10.1101/gad.134205.
8. Goodyer W, Kaitna S, Couteau F, Ward JD, Boulton SJ, et al. (2008) HTP-3 Links DSB Formation with Homolog Pairing and Crossing Over during C. elegans Meiosis. Developmental Cell 14: 263–274. doi:10.1016/j.devcel.2007.11.016.
9. Martinez-Perez E (2005) HTP-1-dependent constraints coordinate homolog pairing and synapsis and promote chiasma formation during C. elegans meiosis. Genes & Development 19: 2727–2743. doi:10.1101/gad.133850.
10. Severson AF, Ling L, van Zuylen V, Meyer BJ (2009) The axial element protein HTP-3 promotes cohesin loading and meiotic axis assembly in C. elegans to form multistranded, cross-striated fibers in vivo. J Cell Biol 142: 331–339. doi:10.1083/jcb.200807089.
11. Yuan L, Brundell E, Hoog C (1996) Expression of the meiosis-specific synaptonemal complex protein 1 in a heterologous system results in the formation of large protein structures. Experimental Cell Research 229: 272–275. doi:10.1006/excr.1996.0371.
12. Goldstein P (1986) The synaptonemal complexes of Caenorhabditis elegans: the dominant him mutant mT6 and pachytene karyotype analysis of the X- autosomal translocation. Chromosoma 93:256–60.
13. Yang F, La Fuente De R, Lee NA, Baumann C, McLaughlin KJ, et al. (2006) Mouse SVCP2 Is Required for Synaptonemal Complex Assembly and Chromosomal Synapsis during Male Meiosis. The Journal of Cell Biology 173: 497–507.
14. Padmore R, Cao L, Kleckner N (1991) Temporal comparison of recombination and synaptonemal complex formation during meiosis in S. cerevisiae. Cell 66: 1219–1226.
15. Yuan L, Brundell E, Hoog C (1996) Expression of the meiosis-specific synaptonemal complex protein 1 in a heterologous system results in the formation of large protein structures. Experimental Cell Research 229: 272–275. doi:10.1006/excr.1996.0371.
16. Yuan L, Brundell E, Hoog C (1996) Expression of the meiosis-specific synaptonemal complex protein 1 in a heterologous system results in the formation of large protein structures. Experimental Cell Research 229: 272–275. doi:10.1006/excr.1996.0371.
17. Goldenstein P (1986) The synaptonemal complexes of Caenorhabditis elegans: the dominant him mutant mT6 and pachytene karyotype analysis of the X-autosomal translocation. Chromosoma 93:256–60.
18. Yuan L, Brundell E, Hoog C (1996) Expression of the meiosis-specific synaptonemal complex protein 1 in a heterologous system results in the formation of large protein structures. Experimental Cell Research 229: 272–275. doi:10.1006/excr.1996.0371.
33. Pick E, Golan A, Zimbrer ZJ, Guo L, Sharaby Y, et al. (2012) The minimal dehydrodyselate core of the COP9 signalosome excludes the Ctn1 MPN- domain. PLoS ONE 7: e134900.

34. Choo YY, Belk BK, Lou JI, Eng J, Leck YC, et al. (2011) Characterization of the role of COP9 signalosome in regulating culin E3 ubiquitin ligase activity. Mol Cell Biol 21: 4706–4715. doi:10.1099/mcb.0.005251-0.

35. Petrokis MD, Deshaies RJ (2005) Function and regulation of culin-RING ubiquitin ligases. Nat Rev Mol Cell Biol 6: 9–20. doi:10.1038/nrm1457.

36. Tatsuzono T, Taguchi H, Ito K (2004) Culin-based ubiquitin ligase and its control by NEDD8-conjugating system. curr protein pepit sci 5: 177–184.

37. Wei N, Serino G, Deng X-W (2008) The COP9 signalosome: more than a protease. Trends in Biochemical Sciences 33: 592–600. doi:10.1016/j.tibs.2008.09.004.

38. Tian L, Peng G, Parant JM, Leventaki V, Drakos E, et al. (2010) Essential roles of Jabi in cell survival, spontaneous DNA damage and DNA repair. Oncogene 29: 6125–6137. doi:10.1038/onc.2010.345.

39. Straatmann JW, Grusomar G (2012) Many jobs for one good cop - the COP9 signalosome. Biochemical development and defense. Planta Sci 185: 146–150. doi:10.1016/j.plantsci.2011.10.004.

40. Dvorostenk S, Djajacev I, Beckendorf SK (2002) CSN/jab1 mutations affect axis formation in the Drosophila oocyte by activating a meiotic checkpoint. Development 129:505–64.

41. Dorsken M, Feigl J, Verbeek A, Rouse L, Falbke M, et al. (2012) Crosslocalization is regulated by the neddylation posttranslational regulatory pathway. PLoS Biol 10: e1001390. doi:10.1371/journal.pbio.1001390.

42. Munde KF, 2006. Deletion Mutants in COP9/Signalosome Subunits in Fission Yeast Schizosaccharomyces pombe Display Distinct Phenotypes. Mol Biol Cell 13: 493–502. doi:10.1091/mbc.05-10-0099.

43. Stratmann JW, Grußomar G (2012) Essential roles of Jab1 in cell survival, spontaneous DNA damage and DNA repair. Oncogene 29: 6125–6137. doi:10.1038/onc.2010.345.

44. Dorsken S, Dajac I, Beckendorf SK (2005) The COP9 Signalosome Promotes Degradation of Cyclin E during Early Drosophila Oogenesis. Developmental Cell 9: 699–711. doi:10.1016/j.devcel.2005.05.006.

45. Koginada G, Weinberg CD, Doss A, Molla G, Serino G, et al. (2012) The Organization of a Csn5-containing Subcomplex of the COP9 Signalosome. J. Biol Chem 287: 42031–42041. doi:10.1042/jbc.M112.387977.

46. Yoshida A, Yoneda-Kato N, Panattoni M, Pardi R, Kato J-Y (2010) CSN5/Jab1 promotes degradation of cyclin E and p21Waf1 in cell survival, spontaneous DNA damage and DNA repair. Oncogene 29: 6125–6137. doi:10.1038/onc.2010.345.

47. Koginada G, Weinberg D, Doss A, Molla G, Serino G, et al. (2012) The Organization of a Csn5-containing Subcomplex of the COP9 Signalosome. Journal of Biological Chemistry 287: 42031–42041. doi:10.1042/jbc.M112.387977.

48. Miller RK, Qadota H, Starck TJ, Mercier KB, Wortham TS, et al. (2009) CSN5, a component of the COP9 signalosome complex, regulates the levels of UNC-96 and UNC-98, two components of Melanes in Caenorhabditis elegans muscle. Mol Biol Cell 20: 3608–3616. doi:10.1091/mbc.E09-03-0208.

49. Smith P, Leung-Chiu WM, Montgomery R, Osborn A, Kuznicki J, et al. (2002) The GLH Proteins, Caenorhabditis elegans P granule Components, Associate with CSN-5 and KGB-1, Proteins Necessary for Fertility, and with YZX-1, a Predicted Cytoskeletal Protein. Developmental Biology 251: 333–347. doi:10.1006/dbio.2002.0832.

50. Choo YY, Boh BK, Lou JJW, Eng J, Leck YC, et al. (2011) Characterization of the role of COP9 signalosome subunits 4 and 5 regulate multiple pleiotropic functions revealed through analysis of hypomorphic csn mutants. Plant Signal Behav 6: 698–896.

51. Dorsken S, Djajacev I, Beckendorf SK (2005) The COP9 Signalosome Promotes Degradation of Cyclin E during Early Drosophila Oogenesis. Developmental Cell 9: 699–711. doi:10.1016/j.devcel.2005.05.006.

52. Dorsken S, Djajacev I, Beckendorf SK (2005) The COP9 Signalosome Promotes Degradation of Cyclin E during Early Drosophila Oogenesis. Developmental Cell 9: 699–711. doi:10.1016/j.devcel.2005.05.006.

53. Ciccarelli FD, Izaurralde E, Bork P (2003) The PAM domain, a multi-protein interaction domain, is conserved in all eukaryotes. Nat Cell Biol 5: 177–184.

54. Li S (2004) A Map of the Interactome Network of the Metazoan C. elegans. Science 303: 548–543. doi:10.1126/science.1094103.
89. Chen Y, Negre N, Li Q, Mieczkowska JO, Slattery M, et al. (2012) Systematic evaluation of factors influencing ChIP-seq fidelity. Nat Meth 9: 609–614. doi:10.1038/nmeth.1985.

90. Zhou Z, Hartwig E, Horvitz HR (2001) CED-1 is a transmembrane receptor that mediates cell corpse engulfment in C. elegans. Cell 104: 43–56.

91. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, et al. (2003) Systematic functional analysis of the Caenorhabditis elegans genome using RNAi. Nature 421: 231–237. doi:10.1038/nature01278.