A Family of Zinc Finger Proteins is Required for Chromosome-Specific Pairing and Synapsis during Meiosis in *C. elegans*

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Summary

Homologous chromosome pairing, synapsis, and recombination are prerequisite for proper chromosome segregation during meiosis. Here we show that each chromosome in *C. elegans* relies on a particular C2H2 Zinc Finger protein to accomplish these tasks. These proteins are all encoded within a tandem gene cluster, which includes the *X*-specific *him-8* gene. Three additional family members are collectively responsible for autosomal segregation. Each of these "ZIM" proteins localizes to a specific region called the meiotic Pairing Center on the corresponding chromosome(s) and associates with the nuclear envelope during early meiotic prophase, suggesting a role analogous to the telomere-mediated meiotic bouquet in other organisms. To gain insight into the evolution of these components, we characterized the orthologous genes in the related nematode species *C. briggsae* and *C. remanei.* This protein family appears to be actively undergoing expansion, suggesting that divergence of their cognate sites on different chromosomes may facilitate homologous interactions.

Keywords: Meiosis, homologous chromosome pairing, synaptonemal complex, meiotic bouquet, zinc finger evolution

Introduction

The cycle of sexual reproduction involves the reciprocal processes of fertilization, which joins two parental genomes, and meiosis, the specialized cell division required to partition diploid genomes to produce haploid gametes. During meiosis, a diploid nucleus undergoes a single round of DNA replication followed by two sequential divisions. At the first division, each chromosome must accurately segregate away from its homologous partner so that the resulting cells receive precisely one copy of each chromosome. Inaccuracy in this process can produce aneuploidy, which usually leads to embryonic lethality or pronounced developmental defects.

To ensure accurate homolog segregation, a series of events occurs during meiotic prophase, the endgame of which is the creation of physical links between homologous chromosome pairs. To achieve this, chromosomes must somehow contact each other and assess each other's identity, and homologous interactions must be preferentially reinforced. This association is usually stabilized by the polymerization of a proteinaceous structure between homologs (the synaptonemal complex, or SC), and later by chiasmata, the physical linkages generated through crossover recombination.

In *C. elegans*, the ability of homologs to carry out these essential preliminary steps and to segregate properly is primarily mediated by a special region near one end of each of the six chromosomes. These *cis*-acting regions are known as "Homolog Recognition Regions" or "Pairing Centers" (PCs). Chromosome segments that are separated from this region by translocation or deletion can no longer recombine efficiently with homologous sequences (McKim et al., 1988; Villeneuve, 1994). These sites have at least two separable activities that promote homologous recombination: they stabilize pairing between homologs and also promote the initiation of synapsis (MacQueen et al., 2005). In addition, these regions mediate a meiotic checkpoint that monitors synapsis between homologous chromosomes (Bhalla and Dernburg, 2005). The molecular basis for these activities remains largely unexplained.

In a recent study, we identified HIM-8 as an essential *trans*-acting component required for *X* chromosome segregation (Phillips et al., 2005). HIM-8 is a zinc finger protein that concentrates specifically at the PC region of the *X* chromosome and appears to tether these sites to the nuclear envelope in meiotic nuclei. Mutations in *him-8* result in *X*-chromosome-specific defects in pairing and synapsis, which leads to a high frequency of male (*XO*) progeny (the "High incidence of males," or Him phenotype). *him-8* mutations had no discernable effect on the segregation of the autosomes. Sex chromosomes often display unique behaviors during meiosis, particularly in the heterogametic sex. In *C. elegans*, the two *X* chromosomes must pair and recombine in hermaphrodites (*XX*) yet the single *X* must also segregate properly as a univalent in males (*XO*). Therefore, it was unclear whether the role of HIM-8 was a special

adaptation of the *X* chromosomes or an activity also required by the five autosomes.

The *him-8* gene is located in an operon with three other highly homologous predicted genes that we have named *zim* (*zinc finger in meiosis*) *-1* (T07G12.6), *zim-2* (T07G12.10) and, *zim-3* (T07G12.11). Here, we define the roles of these genes in meiosis, and demonstrate that each of the three ZIM proteins is required for pairing and synapsis of one or two specific autosome pairs. Moreover, we show that each of the ZIM proteins localizes to the appropriate autosomal PCs and, like HIM-8, is associated with the nuclear envelope during early meiosis. Finally, comparisons among related nematode species revealed that the ZIM/HIM-8 tandem gene cluster has undergone recent changes in copy number. Taken together, these results suggest that the divergence of distinct chromosome-binding factors may enhance the fidelity of homologous interactions during meiosis, a role that could contribute to reproductive fitness and also to reproductive isolation between species.

Results

In a recent paper we showed that HIM-8, a C2H2 zinc finger protein, binds to the *X* chromosome PC during meiosis, and is required both for *X* chromosome synapsis and also to stabilize pairing between the *X* chromosomes (Phillips et al., 2005). It was curious that HIM-8 only affects *X* chromosome segregation, yet is required for meiotic functions that are shared by all the chromosomes; that is, all chromosomes must undergo pairing and synapsis to segregate properly in *C. elegans.* This raised the question of whether there might exist autosomal counterparts to HIM-8 that mediate pairing and synapsis.

Genome annotations based on gene predictions and cDNA evidence suggested that *him-8* is one of four highly related genes present in a tandem array that are co-expressed as an operon (Figure 1A), (Blumenthal et al., 2002). The four genes share similar C-terminal regions encoding two noncanonical C2H2 zinc fingers. They also share strong similarity in their N-terminal portions, which lack obvious structural motifs but contain numerous potential modification sites. Based on their similarity to each other and the analysis described here, we have named these genes *zim-1*, *-2*, and *-3* (for "*zinc finger in meiosis*"); the numbering simply reflects their order within the operon.

Due to their extensive similarity, we could not obtain unequivocal functional information about the individual genes by RNAi-mediated knockdown. However, deletion alleles of all three genes, *zim-1(tm1813)*, *zim-2(tm574), zim-3(tm756 and tm2303)*, have been isolated by the Japanese National Bioresource for *C. elegans* (Figure 1A). To gain further insight into the mechanism of homolog pairing and synapsis, we have studied the effects of these mutations on meiotic chromosome behavior. We also raised antibodies specific for each of the three ZIM proteins to investigate their localization during meiosis.

Mutations In The *zim* Genes Result In Meiotic Defects

Deletions in each of the three *zim* genes were isolated by PCR screening of randomly-mutagenized worm libraries. Worms homozygous for each of the deletions were morphologically normal and fertile but gave rise to broods containing both inviable progeny (embryos that fail to hatch) and an elevated incidence of males. Taken on its own, embryonic inviability is a fairly uninformative phenotype, since it can result from anueploidy due to chromosome missegregation during meiosis but can also reflect loss of any one of myriad activities required for early development. However, the elevated incidence of male progeny, known as the "Him" phenotype, is diagnostic for meiotic *X* chromosome segregation defects. We quantified the frequency of males and inviable progeny (Figure 1B) and found that *zim-1* and *zim-3* were quantitatively very similar but *zim-2* mutants produced substantially fewer males and more viable progeny than either of the other mutants. Mutations in *him-8*, which abrogate *X* chromosome pairing and synapsis, result in ~40% male progeny and nearly 100% viable embryos (Phillips et al., 2005). These data indicate that segregation of the *X* chromosome does not depend as strongly on *zim-1, -2,* or *-3* as it does on *him-8*. On the other hand, the high progeny inviability indicates suggested that the ZIM proteins may be important for autosomal segregation.

To determine whether the extensive inviability among the progeny of *zim* mutants arises from segregation defects during meiosis, we directly examined chromosome behavior during meiotic prophase. To evaluate synapsis, we performed immunofluorescence detection of two components of the SC. HTP-3 is an axial component and localizes to meiotic chromosomes prior to synapsis, while SYP-1 is a central element protein that loads during synapsis (MacQueen et al., 2002; MacQueen et al., 2005). By definition, chromosomes or chromosome segments that load HTP-3 but not SYP-1 are unsynapsed. By this assay, *zim-1, zim-2,* and *zim-3* mutants consistently exhibited unsynapsed chromosomes in their pachytene-stage nuclei (Figure 1C-F). This finding clearly implicates each of the *zim* genes in homolog pairing and/or synapsis during early prophase.

Another diagnostic assay for defects in homologous chromosome interactions is visual examination of chromosomes at the diakinesis stage of meiosis, which occurs near the end of prophase shortly before the first meiotic division. In wild-type hermaphrodites the six homologous chromosome pairs are held together at this stage by chiasmata. Accordingly, six DAPI-stained bodies, or bivalents, corresponding to the five autosome pairs and single pair of sex chromosomes, are normally observed. Nonrecombinant, or achiasmate, chromosomes are detected as extra DAPI-stained bodies called univalents at diakinesis. This assay is less specific than direct visualization of homolog synapsis, since univalents can result from defects in pairing, synapsis, recombination, or cohesion, but the number of DAPI-stained bodies can be quantified more precisely than unsynapsed chromosomes. Most nuclei in *zim-2* mutants contained seven DAPI-stained bodies, representing five bivalents and

two univalents, whereas *zim-1* and *zim-3* mutants most frequently had eight DAPI-stained bodies (Figure 2A, B).

Mutations that eliminate synapsis or recombination in *C. elegans* typically result in detection of 12 univalent chromosomes at diakinesis and survival of fewer than 5% of embryonic progeny. The survivors presumably reflect a low frequency of zygotes with viable chromosome complements arising by random segregation of six chromosome pairs. The frequency of both bivalents and viable progeny in each of the *zim* mutants is therefore inconsistent with a complete failure in chromosome synapsis. The fact that four or five chromosomes successfully undergo exchange in each oocyte could indicate that the ZIM proteins act combinatorially to promote synapsis, and that loss of any one of their functions reduces the probability of synapsis of each chromosome pair to about 2/3 in *zim-1* and *zim-3* and 5/6 in *zim-2*. However, if each chromosome were affected in a probabilistic way by the mutations, we would expect to see a broader frequency distribution of nonrecombinant chromosomes at diakinesis. The tight distribution in each of the *zim* mutants is more consistent with the idea that each of the mutations specifically or preferentially impairs synapsis of a subset of the autosomes.

Each Zim Gene Is Responsible For Synapsis Of Specific Autosome Pairs

To identify which chromosomes were affected by each of the *zim* mutations, we performed FISH using probes specific for each of the six chromosomes to assess pairing at the pachytene stage, where all chromosomes are normally paired and synapsed. In *zim-2* hermaphrodites, a probe to chromosome *V* consistently displayed two unpaired FISH signals at pachytene, while probes to all other chromosomes were paired. By contrast, in the *zim-1* and the *zim-3* mutants, probes to two different chromosome pairs, *II* and *III* for *zim-1* and *I* and *IV* for *zim-3*, were abnormally separated at pachytene (Figure 2C-F). In all *zim* mutants, the *X* chromosomes synapsed normally. While there may be subtle effects on the *X* chromosome that are not easily detected cytologically, it is also possible that the elevated frequencies of *X* missegregation observed in the *zim* mutants is an indirect effect of autosomal asynapsis. We will return to this issue in a later section of the Results.

This analysis clearly implicates each of the *zim* genes in the segregation of either one or two autosome pairs. Conversely, it reveals that each of the five autosomes is acutely affected by one and only one of the *zim* genes. *zim-2* affects only one chromosome pair while *zim-1* and *zim-3* mutations each perturb two autosomes. This agrees well with the numbers of bivalents and univalents seen at diakinesis in each of the mutants, and can also explain the greater embryonic lethality observed for *zim-1* and *zim-3*.

As meiosis initiates, chromosomes adopt a polarized organization within meiotic nuclei and the nucleolus is displaced towards one side (MacQueen and Villeneuve, 2001). Once pairing and synapsis are accomplished, chromosomes are normally redistributed around the nuclear periphery. As previously observed for *him-8* mutants (Phillips et al., 2005), *zim-1*, *zim-2,* and *zim-3* mutant

hermaphrodites showed a delayed exit from this polarized state (Figure 6A, top panel). Such delays are also associated with other synapsis defects and reflect the persistence of recombination intermediates on unsynapsed chromosomes (Carlton et al., 2006). The perdurance of polarized nuclei in the *zim* mutants implies that this delay can be triggered by asynapsis of any pair of chromosomes.

Pairing of Chromosome *V* Is Disrupted in the *zim-2* Mutant

To ascertain whether *zim* mutants are defective in homolog pairing or specifically fail to initiate synapsis, we analyzed the dynamics of homolog pairing in *zim-2* mutants by FISH. Homologous interactions were quantified as a function of prophase progression by dividing the gonads of age-matched animals into sequential intervals (Figure 3A). The most distal zone includes premeiotic nuclei, which are undergoing mitotic proliferation. Zone 2 includes mostly nuclei at the leptotene/zygotene stage, during which homologs pair and initiate synapsis. Zones 3-5 represent the pachytene region, where chromosomes are normally fully aligned and synapsed with their homologs.

Fluorescent probes to the left and right ends of chromosome *V* were hybridized to dissected wild-type and *zim-2* animals. A probe that hybridizes to the center of the *X* chromosome was included for comparison (Figure 3B). The fraction of nuclei containing paired FISH signals was measured for each zone. In wild-type control animals, all three FISH probes showed only infrequent homolog associations in Zone 1, but in Zone 2 paired FISH signals began to appear, as previously described (MacQueen et al., 2002; Phillips et al., 2005). By Zone 3, synapsis is normally complete, as detected by nearly 100% pairing of all probes. In *zim-2* hermaphrodites, pairing of the *X* chromosome proceeded normally, but pairing of chromosome *V* was severely compromised, as reflected by the observation that neither probe ever showed a frequency of pairing above the background level seen in the premeiotic region (Figure 3C,D).

In the absence of synapsis, pairing at the PC ends of chromosomes is stabilized relative to pairing at non-PC ends, an activity that requires interaction between homologous PCs (MacQueen et al., 2002; MacQueen et al., 2005). Synapsis-independent stabilization of pairing at the PC of the of *X* chromosome depends on HIM-8 function (Phillips et al., 2005). Similarly, in the *zim-2* mutant, no stabilization is seen at the PC end of chromosome *V*. These data indicate that ZIM-2, like HIM-8, is required both for synapsis-independent stabilization of pairing and for synapsis of a specific chromosome pair and that the stabilization of *X* chromosome pairing does not involve a unique mechanism.

ZIM Proteins Bind to the Pairing Centers of the Autosomes They Control

To study the localization of the ZIM proteins, polyclonal antisera were raised against synthetic peptides to nonconserved regions (see Experimental Procedures). Each of the three antisera localized to discrete chromosome-associated foci that were most prominent in the transition zone and very early pachytene (Figure 4A). At the onset of meiosis, ZIM-2 was detected at no more

than two foci. By early pachytene, shortly before the signals disappeared, ZIM-2 was consistently detected as a single focus in each nucleus (Figure 4C). By contrast, ZIM-1 and ZIM-3 often appeared to localize to 3-4 foci at the onset of meiosis, and by early pachytene were consistently observed at two foci (Figure 4B, D).

Based on the knowledge that HIM-8 concentrates at the *X* chromosome Pairing Center, we considered it highly likely that the foci of ZIM-1, -2, and -3 proteins corresponded to autosomal PCs, and most probably to the chromosomes that missegregate in the corresponding mutants. To test this, we combined immunofluorescence detection of each ZIM protein with FISH to localize the PC region of each autosome. Indeed, these experiments revealed clear correspondence of ZIM-1 with the left ends of both chromosome *II* and chromosome *III* (Figure 5A-D), ZIM-2 with the right end of chromosome *V* (Figure 5E-H), and ZIM-3 with the right end of chromosome *I* and the left end of chromosome *IV* (Figure 5 I-P). Taken together, these observations indicate that the ZIM proteins are functional paralogs of HIM-8 that bind to the PCs of specific chromosomes and are required on those chromosomes to mediate meiotic chromosome pairing and synapsis (Figure 5Q).

ZIM Proteins Localize To Chromosomes Independently Of Each Other And Most Other Known Meiotic Components

A striking difference between the three ZIM proteins and HIM-8 is the timing of their appearance and disappearance. HIM-8 is clearly detected in premeiotic germline nuclei and staining persists through the end of pachytene (Phillips et al., 2005). By contrast, the ZIMs do not appear as distinct foci until the onset of meiosis, and they disappear as soon as their known functions are accomplished, at the completion of chromosome pairing. Interestingly, mutations that cause a delay in the disappearance of polarized, early pachytene nuclei, including the *zim* and *him-8* mutants themselves, *rec-8(ok978), and syp-1(me17),* do not affect localization of ZIM proteins to the autosomes, but do result in perdurance of staining throughout the region of polarized nuclei (Figure 6A, B and data not shown). This implies that the disappearance of ZIM proteins from the PCs is coupled to the loss of nuclear polarization, but does not temporally correspond to the completion of synapsis by the individual chromosomes to which they are bound. Differences in the timing of appearance and disappearance of HIM-8 vs. ZIM proteins may reflect either transcriptional or posttranscriptional regulation, but the latter seems more likely given that the four genes are in the same operon and are thus likely cotranscribed.

We performed ZIM immunofluorescence in a number of meiotic mutants affecting the recombination machinery, axial or central SC components, cohesins, or regulatory factors [*chk-2(me64), him-3(gk149), htp-1(gk150),* and *zhp-3(jj61)*, in addition to those mentioned above]. Only one mutation affected ZIM protein staining: *chk-2(me64)*. Distinct ZIM foci are not detected in *chk-2(me64)* animals, which lack a transition zone and show profound defects in chromosome pairing (MacQueen and Villeneuve, 2001). By contrast, HIM-8

localization to the *X* chromosome is retained in *chk-2* mutants (Phillips et al., 2005). This provides further evidence that the ZIMs and HIM-8 may be controlled by distinct, likely posttranslational, regulatory mechanisms, but the significance of this finding is unclear.

Pairing Centers Associate With The Nuclear Envelope But Do Not Cluster

Like HIM-8, all ZIM protein foci were consistently observed at the nuclear periphery, suggesting a direct association with the nuclear envelope. This association was even more evident in samples that were stained with for both ZIM proteins and the nuclear lamina (Figure 4E-G). This observation indicates that all PCs are likely associated with the nuclear envelope during the stages of homolog pairing and synapsis initiation.

During meiotic prophase in diverse organisms, telomeres associate with the nuclear envelope and cluster together transiently in a configuration known as the meiotic bouquet (reviewed by Scherthan, 2001). We therefore tested whether the twelve chromosomal PCs, as detected by the four ZIM/HIM-8 antibodies, cluster in meiotic nuclei. Gonads were stained with all compatible combinations of antibodies (Figure 6B and data not shown). The staining of each ZIM protein was not correlated with other ZIMs or with HIM-8. When hermaphrodites were co-stained with all three ZIM antibodies and the HIM-8 antibody, several dispersed foci were detected at the periphery of each nucleus, further indicating that these sites do not concentrate in one region of the nuclear envelope (Figure 6C).

We considered the possibility that clustering of PCs might occur transiently during homolog pairing. To test this idea, we looked at HIM-8 and ZIM protein foci in synapsis mutants that cause an extended region of polarized nuclei, including *syp-1* and the *zim* mutants, with the idea that these situations might prolong a normally transient clustering phase. However, clustering was not observed even in these situations (data not shown). Although we do not detect clustering by immunofluorescence, it remains possible that transient associations among PCs might be revealed by dynamic analysis.

Meiotic Checkpoints Contribute To The Rates Of Missegregation Observed In ZIM Mutants

In a previous study we proposed that the stabilization of pairing by the PCs may provide a “kinetic proofreading” mechanism to facilitate accurate assessment of homology before synapsis (MacQueen et al., 2005). To test this idea, we combined the *zim-2* mutation with a deficiency of one copy of the *X* chromosome PC (*zim-2; meDf2*/+). If synapsis in the absence of PC-mediated stabilization of pairing is more promiscuous with respect to partner choice, we expected to detect some nonhomologous synapsis between the *X* chromosome that retains its PC and either copy of chromosome *V*. Indeed, we observed rare examples of this configuration but synapsis between *X* chromosomes occurred much more frequently (data not shown), indicating that homologous synapsis is strongly favored even when there is no PC-mediated stabilization of pairing.

To quantify the extent to which the presence of unsynapsed chromosome *V* might impede the ability of *X* chromosomes to interact when only one of them has a PC, we measured the frequency of male production. Heterozygous *X*-chromosome PC deficient hermaphrodites of the genotype *meDf2*/+ produce an average of 7% males (Bhalla and Dernburg, 2005), and *zim-2* alone results in 1.3% male self-progeny (above). The combination of *zim-2; meDf2*/+ resulted in 13% male self-progeny, more than an additive effect of the two mutations.

Initially, we interpreted this synergism to mean that the unsynapsed autosomes were substantially interfering with *X* chromosome synapsis by direct competition. However, this interpretation does not take into account the possible effects of germline apoptosis. At least two meiotic checkpoints in *C. elegans* that can eliminate a substantial fraction of oocytes with unrepaired recombination intermediates or unsynapsed chromosomes. Normally the synapsis checkpoint preferentially removes nuclei with unsynapsed *X*s in a *meDf2*/+ heterozygote, resulting in a frequency of male production far below the frequency of unsynapsed chromosomes (Bhalla and Dernburg, 2005). The presence of unsynapsed autosomes in every nucleus due to the *zim-2* mutation should also trigger apoptosis, and this might markedly reduce the ability of checkpoints to selectively cull nuclei with unsynapsed *X* chromosomes. To control for this effect, we repeated the quantitative analysis of male production in the absence of apoptosis, by introducing a *ced-4* mutation into the strains.

There was no significant difference between the number of male self-progeny produced by *ced-4; meDf2*/+ hermaphrodites (19.4%) and *ced-4; zim-2; meDf2*/+ hermaphrodites (21.5%). This indicates that the apparent synergy between the *zim-2* mutation and the loss of a single *X* PC is fully accounted for by the effects of apoptosis, and therefore does not reflect competition for synapsis of the *X*s. Although these findings did not support our hypothesis that competition for synapsis between non-homologous and homologous chromosomes might significantly impair homologous chromosome synapsis, it does underscore the significant impact that meiotic checkpoints can contribute to experimental results.

In particular, this finding can help to explain the nondisjunction of *X* chromosomes that we detect in the *zim* mutants as an elevated frequency of male progeny (Figure 1B). We favor the idea that this Him phenotype may reflect indirect consequences of autosomal asynapsis rather than a direct effect of absence of the ZIM proteins. Mutations that eliminate germline apoptosis in *C. elegans* result in the production of 1-2% male progeny (Bhalla and Dernburg, 2005). This suggests that about 1-2% of oocytes normally have unsynapsed or nonrecombinant *X* chromosomes, and that most of these are normally eliminated by meiotic checkpoints to result in a 10-fold lower frequency of male self-progeny. *zim-2* mutants produce 1.3% males, which can be explained if the presence of unsynapsed autosomes in most oocyte nuclei saturates the checkpoints or apoptosis machinery, or otherwise renders the unsynapsed Xs invisible to selective elimination mechanisms. The higher frequencies of males produced by *zim-1* and *zim-3* mutants are somewhat harder to explain. FISH in

these mutants has not indicated any increase in unsynapsed *X* chromosomes. However, it is possible that the unsynapsed autosomes reduce the efficiency of *X* chromosome synapsis. Alternatively, the presence of multiple univalents during the MI division may perturb chromosome congression or the ability of the meiotic spindle to accurately segregate properly recombined *X* bivalents.

Evolution of the ZIM/HIM-8 Protein Family

Because *zim-1*, *zim-2*, *zim-3*, and *him-8* share extensive structural and functional similarities and are organized as a tandem gene array, they have likely arisen from a common ancestor through gene duplication and selection. To learn more about the evolution of this family of proteins, we identified and characterized homologous genes in two related nematode species, *C. remanei* and *C. briggsae*, for which extensive genome sequence is available. In each case homology searches identified one or more contigs that encode apparent orthologs of the *C. elegans* genes, but some gap-filling was required to complete and annotate the sequence of the syntenic regions (see Experimental Procedures).

This analysis revealed the presence of four ZIM/HIM-8 genes in *C. remanei* and five in *C. briggsae* (Genbank Accession number DQ498827 and Supplementary Data). In both species, as in *C. elegans*, all genes are closely spaced in a tandem array, suggesting that their operon organization is conserved (Figure 7A). The difference in gene number does not reflect a difference in the number of chromosomes. Visualization of bivalents at diakinesis revealed that all three species have six chromosome pairs (Figure S1A, B, D).

To evaluate the relationship among the ZIM/HIM-8 proteins in the three nematodes, we used ClustalX (Chenna et al., 2003; Thompson et al., 1994). Specifically, we investigated whether there were clear orthologous relationships between the genes in different species and whether the order of genes in the tandem array was conserved.

Sequence comparisons revealed blocks of strong conservation in the N- and C-terminal portions of the proteins, including the Zn finger region in the C-terminal half. We generated separate trees using the predicted amino acid sequences of the two conserved domains using the neighbor-joining method. These are displayed in Figure 7C as unrooted trees generated by TREEVIEW (Page, 1996). The C-terminal regions, containing the two Zn fingers, showed greatest conservation with the corresponding proteins in the other two species, but the N-termini were more closely related to the other ZIM/HIM-8 proteins within the same species. We hypothesize that the Zn fingers have retained their individuality as distinct sequence-binding motifs, while the N-termini within each species are likely co-evolving with a common set of interacting proteins.

Based on sequence homology, the downstream gene in each operon is likely orthologous to *him-8*. There was some ambiguity about this prediction in *C. briggsae*, both because of greater sequence divergence from *C. elegans* and

also because *C. briggsae* appears to have an extra gene relative to the other two species. In some respects the extra gene resembles *him-8* from *C. elegans*.

To obtain functional information about the *C. briggsae* genes, we carried out RNAi to inactivate both the last gene in the operon and the gene we identified as a unique gene based on its divergence from the *C. elegans* and *C. remanei* proteins. Double-stranded RNA corresponding to regions of these genes sharing minimal nucleotide sequence similarity with other family members was injected into adult *C. briggsae* hermaphrodites (see Experimental Procedures). Progeny of the injected animals were analyzed for meiotic chromosome segregation defects, particularly an increase in the incidence of males among their offspring (i.e., males in the F2 generation). In addition, we examined the number of DAPI-stained bodies at diakinesis among the F1 generation.

Inactivation of the downstream gene in *C. briggsae* by RNAi resulted in F1 animals with a strong Him phenotype. We saw a high variance in the frequency of male production, presumably due to the variability inherent in RNAi experiments, but the most severely affected animals produced 22-31% males (Figure S1D,E). Cytological analysis revealed that *him-8*^{RNAi} F1 animals usually had seven DAPI-stained bodies at diakinesis, indicating a single pair of non-recombinant chromosomes (Figure S1D,F). Together these data indicate that this gene specifically affects *X* chromosome behavior, corroborating the identification of this gene as *Cb-him-8*.

We did not observe meiotic defects following RNAi of the gene we have named *Cb-zim-4*. Nuclei from *zim-4*^{RNAi} hermaphrodites usually had six DAPI-stained bodies at diakinesis (Figure S1D,F), as did uninjected controls, indicating that all chromosome pairs efficiently underwent pairing, synapsis, and crossing-over. These results may indicate that the gene lacks an essential role in meiotic pairing and synapsis, perhaps because it has diverged recently and shares functional redundancy with another *C. briggsae* gene. Alternatively, we may not have effectively knocked down the function of this gene by RNAi.

Discussion

Together with a previous study (Philips et al., 2005), the experiments presented here show that each chromosome in *C. elegans* requires the activity of a specific member of the ZIM/HIM-8 protein family to mediate efficient synapsis, recombination, and ultimately segregation. These proteins localize to the genetically-defined PC regions of the corresponding chromosomes, perhaps by binding to specific sequences enriched within these regions. Like HIM-8, ZIM-2 localizes to a single pair, chromosome *V*, while ZIM-1 and ZIM-3 each localize to two chromosome pairs.

It is not obvious why some chromosomes have unique PC-binding proteins yet two pairs of autosomes each share a single protein. We have tested whether nonhomologous chromosomes that share a ZIM protein, i.e. chromosomes *II* and *III* or *I* and *IV,* undergo appreciable levels of non-homologous synapsis, but we have never observed such a configuration in wild-

type animals (data not shown). Along with prior evidence, this indicates that the specificity of homolog recognition cannot be solely defined by either by the role of the PCs or the identity of the corresponding ZIM/HIM-8 family member.

Analysis of ZIM/HIM-8 homologs in related species reveals that 1) different domains of the proteins show distinct patterns of conservation and divergence within and between the species and 2) the number of genes in the family is not static, and is likely increasing. The second conclusion is based on knowledge that *C. briggsae* and *C. remanei* are more closely related to each other than either species is to *C. elegans* (Kiontke et al., 2004). It is therefore more parsimonious to propose that the extra *zim* gene in *C. briggsae* has resulted from a recent gene duplication than independent instances of gene loss in *C. remanei* and *C. elegans.*

It seems most likely that the ZIM/HIM-8 family in *C. elegans* represents an intermediate state in evolution. We imagine that there was originally a single member of the ZIM/HIM-8 family that bound to all chromosomes, but the duplication and divergence of the protein family (in concert with their binding sites) gradually enhanced either the speed or accuracy of the process of meiotic pairing and synapsis. The modular structure zinc finger proteins, which often have separate regulatory domains and sequence-specific DNA-binding domains, allows for rapid acquisition of new binding specificities without perturbing regulation or interactions with binding partners (Shannon et al., 2003). We predict that a distinct PC-binding protein for each of the six chromosomes would be a more optimal situation, and that *C. briggsae*, with five family members, may be one step closer to this condition than *C. elegans* or *C. remanei*.

In other species, chromosome attachment to the nuclear envelope during meiosis is mediated by telomeres. In *S. pombe* and *S. cerevisiae* this process requires telomere-binding proteins shared by all chromosomes: Ndj1 in budding yeast and a complex including Rap1p, Taz1p, Bqt1p, and Bqt2p in fission yeast (Chikashige and Hiraoka, 2001; Chikashige et al., 2006; Cooper et al., 1998; Nimmo et al., 1998; Trelles-Sticken et al., 2000). To date, there are no known chromosome-specific factors required for participation in the meiotic bouquet. It is likely that PCs in *C. elegans* mediate the same function(s) during meiosis as bouquet formation, and that the ZIM/HIM-8 family link the chromosomes to the nuclear envelope by binding directly to the DNA.

The process of meiotic pairing and synapsis seems to be much more highly dependent on PCs in *C. elegans* than it is on bouquet formation in budding yeast. This may reflect the greater genome complexity of higher eukaryotes, and a corresponding need to ensure global chromosome alignment prior to the onset of DNA homology-based search mechanisms. It would be interesting to analyze the consequences of bouquet disruption in other metazoans or higher plants, but other than our studies of the ZIM/HIM-8 family in *C. elegans* there are no reports outside of single-celled fungi of mutations that specifically impair telomere organization or nuclear envelope association of meiotic chromosomes.

Experimental Procedures

Genetics and mutant alleles

The *C. elegans* wild-type strain, N2 Bristol, and all other strains were cultured under standard conditions at 20˚C (Brenner, 1974). Mutant alleles of the three *zim* genes were generated and provided by the Japanese National Bioresource Project. One *zim-3* allele, *tm756,* is an in-frame deletion that disrupts the first of two zinc fingers. The other deletions, including *tm574*, *tm1813*, and *tm2303,* cause frame shifts that result in early stop codons. Based on sequence analysis, all of these alleles are expected to result in complete loss-of-function of the corresponding gene, and this is supported by our phenotypic analysis, described in the Results section. All of the deletion mutations were found to be fully recessive; that is, no meiotic defects were detected in *zim*/+ heterozygotes. All mutations were outcrossed at least five times before analysis. Phenotypic analysis of all mutations was carried out using homozygous mutant progeny from heterozygous parents, to ensure that meiosis in the preceding generation was unperturbed and that the animals we analyzed therefore carried a euploid chromosome complement.

We were initially unable to generate adult animals that were homozygous for either of two deletion alleles of *zim-3 (tm756 and tm2303).* Homozygotes for either mutation died as embryos or larvae, precluding analysis of their meiotic chromosome behavior. Because of this lethality, we initially suspected that ZIM-3 might play a more general role in development than the other ZIM/HIM-8 proteins. We tested this idea by generating animals that were heterozygous for *zim-3(tm756)* and deficiencies of the corresponding region of chromosome *IV* (*mDf7 and sDf2)*. These *zim-3(tm756)/Df* combinations produced viable, fertile adults, as did the trans-heterozygous combination of the two *zim-3* deletion alleles *(tm756/tm2303)*. This indicates that the two *zim-3* alleles are associated with distinct lethal mutations, and that the lethal mutation in the *zim-3(tm756)* strain is complemented by both of the chromosome *IV* deficiencies we tested. While *tm756* appears to be associated with a closely-linked chromosomal aberration that could not be separated from the deletion by recombination, we were able to separate *zim-3*(*tm2303)* from a linked lethal mutation. From heterozygotes of the genotype *zim-3(tm2303)/unc-24 dpy-20 (IV),* we recovered an Unc non-Dpy recombinant that carried *tm2303* and produced fertile, homozygous *unc-24 zim-3(tm2303)* progeny. The genetic analysis reported here was obtained from the resulting strain. Cytological analysis was performed with both the *unc-24 zim-3(tm2303)* and the trans-heterozygous *zim-3(tm756/tm2303)*, supporting the idea that both alleles result in loss of *zim-3* function, but the latter animals were not well-suited to genetic analysis due to the inviability of their *zim-3(tm756)* progeny.

In addition to the *zim* mutant alleles characterized in detail in this paper, we examined one other deletion in this region, T07G12.8(*tm1479*). This mutation

removes a segment of a large intron of the *zim-1* gene. This deletion did not result in complete loss of ZIM-1 expression, as judged by immunofluorescence, but did result in detectable synapsis defects of chromosomes *II* and *III*.

Antibodies

To raise polyclonal antibodies specific to each of the three ZIM proteins, peptides corresponding to unique, predicted antigenic regions of each protein were synthesized and coupled to KLH. ZIM-1: IGPVRKAERTPRRKLKSIRL; ZIM-2: GKPRRYKKCKNSLKNTPEVDNENVDKDS; and ZIM-3: SRQDKGSKRSQKSMDSGAKQKLDEARDED. Rabbits and/or guinea pigs were immunized with each peptide. Crude antisera were used for all experiments reported here, except for ZIM-3, which was affinity purified against the ZIM-3 peptide. ZIM-1 serum was preadsorbed against formaldehyde-fixed wild-type worms to reduce non-specific staining.

Fluorescently-conjugated secondary antibodies were purchased from Jackson ImmunoResearch or Molecular Probes.

Immunofluorescence and FISH

For DAPI staining and immunofluorescence, dissected gonads were fixed in 3.7% or 1% formaldehyde, respectively, in Egg Buffer containing 1% Tween-20, freeze-cracked into cold 100% dimethylformamide or ethanol, and then washed in PBS with 0.1% Tween-20 (Dernburg et al., 1998).

FISH experiments were performed as in (Dernburg et al., 1998), except that that a variable wattage-microwave with circulating waterbath (Biowave, Ted Pella) was used to accelerate hybridization. Pairing analysis was performed using age-matched adult worms, 20-24 hours post-L4 larval stage. Gonads were divided into 5 evenly spaced intervals from beginning at the distal tip through the end of the pachytene region. FISH signals were considered paired if they were within 0.7 μm of each other (MacQueen et al., 2002).

The probes used for *zim-2* pairing analysis were as follows. The VR probe was synthesized from a pool of cosmids: T08C12, F26F2, W08A7, F46B3 and W01F3. VL was described by (MacQueen et al., 2005) and was made from a pool of cosmids: T27A9, T25C8, T12D8, and ZK526. The XC probe was a synthetic oligonucleotide of the sequence TTTCGCTTAGAGCGATTCCTTACCCTTAAATGGGCGCCGG, which is highly enriched on cosmid C07D8 (Lieb et al., 2000; Phillips et al., 2005). IIL (from Figure 2) and IIIL were made from cosmid pools F43C11, F53G2 and F59H5 (for IIL) and T19C3, K02F3, and W02B3 (for IIIL). IVC was made from a pool of four cosmids flanking but not including the *spo-11* gene. VR (from Figure 2) was made from the 5s rDNA repeat and was described by Dernburg et al. (1998).
IIL (from Figure 6), IR, and IVL were single fosmids: 33cD05, 37aC10, and 11cC03, respectively.

Most FISH probes were labeled by 3' tailing with aminoallyl-dUTP followed by conjugation to Cy3-NHS-ester, Cy5-NHS-ester (Amersham/GE) or Alexa 488 or Oregon Green 488 succinimidyl-esters (Molecular Probes). Fosmid probes

were 3'-end-labeled with digoxigenin-dUTP (Roche) and detected with fluorescent anti-digoxin antibodies (Jackson ImmunoResearch).

C. remanei and *C. briggsae* Sequencing and Gene Annotation

Using BLAST analysis, we determined that the genomic region of *C. remanei* with homology to the *C. elegans* ZIM/HIM-8 operon is contained within supercontig6 of the 8/2005 preliminary release of the *C. remanei* genome. The published sequence contains three gaps, spanning Contig6.71, Contig6.72, Contig6.73, and Contig6.74. We designed primers to amplify across the gaps: GTGGTCTCGTTCAAAGTTCC and CATTTGACCGACAGTTTGGC for the first gap, AAACTTCAGTCATTCCGACTG and ATACCACGATCAACTTTCGTG for the second gap, and AGCAGCTCAGAAAACTACCC and GCTCTTCATTGAATGCATCC for the third gap. These regions were amplified by PCR using Bio-*X*-Act (Bioline) enzyme and *C. remanei* DNA as a template and sequenced. The sequences of the PCR products are included as Supplemental Data, along with the sequence if the resulting contiguous region.

A BAC clone (RPCI94_13P22) spanning the syntenic region of *C. briggsae* was obtained from the BAC/PAC Resource Center. It was subcloned, shotgun sequenced, and assembled (Genbank Accession #DQ498827).

To identify the *zim* and *him-8* genes in *C. briggsae* and *C. remanei*, we used the combined outputs of Genscan, GenomeScan and GeneWise, along with manual editing to define ambiguous intron/exon boundaries, usually by bringing the paralogs into register (Birney et al., 2004; Burge and Karlin, 1997; Yeh et al., 2001). Where we saw discrepancies between the sequenced PCR products and the published nucleotide sequences from *C. remanei*, we used our experimentally-determined sequences for gene annotations. Predicted cDNAs are included as Supplementary Data (for *C. remanei*) and as GenBank Accession #DQ498827 (for *C. briggsae*).

C. briggsae RNAi

Double-stranded RNA to inactivate the predicted *C. briggsae him-8* and *zim-4*, genes was synthesized from PCR products generated with the following primers: TGCAATTTAGAAGTTCCGCG and GGATAGGAATTGTAATCTCGC for *Cb-him-8* and CAAGTGAATATTTACGGGCG and CATCTGACGATTTTTCAGACC for *Cb-zim-4*. The T7 promoter sequence (TAATACGACTCACTATAG) was added to the 5' end of each primer so that double-stranded RNA could be directly synthesized from PCR products using the MEGAscript High Yield Transcription Kit (Ambion). PCR using *C. briggsae* genomic DNA as a template was carried out using Bio-*X*-Act enzyme. Double-stranded RNA was transcribed *in vitro* from the PCR products using the MEGAscript High Yield Transcription Kit (Ambion). RNA was analyzed on a 1% agarose gel to verify size and integrity.

Adult *C. briggsae* hermaphrodites were injected as young adults, approximately 12 hours after the L4 larval stage. Injected animals were kept at 15°C for 20 hours and then transferred individually to new plates. Their F1

progeny were later transferred to individual plates and scored for the RNAi phenotype by brood analysis and DAPI staining.

Acknowledgements

We are extremely grateful to Shohei Mitani and the Japanese National Bioresource for isolating and providing the deletion mutants described here. We also thank John Spieth, the BACPAC Resource Center, and especially Jan-Fang Cheng for providing BAC information and sequencing. Fosmids used for FISH probes were generated by the *C. elegans* Reverse Genetics Core Facility in Vancouver and graciously provided by Jaryn Perkins and Don Moerman. The SYP-1 and LMN-1 antibodies were generous gifts from Anne Villeneuve and Yossi Gruenbaum, respectively. We thank Gary Karpen for advice on the manuscript.

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Figure Legends

Figure 1. Deletion of *zim-1*, *zim-2,* or *zim-3* Results in Meiotic Chromosome Missegregation and Unsynapsed Chromosomes.
(A) Schematic of the ZIM/HIM-8 operon indicating the region of each gene removed by the deletion alleles described here. *zim-3*(*tm756)* is an in-frame deletion, while *zim-1(1813), zim-2(tm574),* and *zim-3*(*tm2303)* result in frame shifts and early stop codons.
(B) Frequencies of males and viable embryos observed among whole broods in wild-type, *zim-1, zim-2 ,* and *zim-3* hermaphrodites.
(C-F) Pachytene nuclei showing immunofluorescence of SYP-1 (green) and HTP-3 (red) for of each indicated genotypes.
(C) Wild-type nuclei display extensive colocalization of SYP-1 and HTP-3 along the lengths of all six chromosomes.
(D-F) In *zim-1, -2, and -3* mutants, unsynapsed chromosomes are detected at pachytene as segments that stain with HTP-3 (red) but not SYP-1 (green). Arrowheads indicate particularly clear examples of unsynapsed chromosomes.
All images are projections of deconvolved 3D images. Scale bars represent 5μm.

Figure 2. *zim* Mutants Display Chromosome-Specific Defects in Synapsis and Chiasma Formation
(A) Wild-type nuclei have six DAPI-stained bodies at diakinesis, representing the six pairs of homologous chromosomes held together by chiasmata. The number of DAPI-stained bodies can be underestimated if two pairs of chromosomes are too close to one another to be visually resolved. The histogram indicates the distribution of oocyte nuclei with various numbers of DAPI-stained bodies is for wild-type hermaphrodites and the three *zim* mutants. (B) The mean number of DAPI-stained bodies detected for the same genotypes shown in (A). Examples of a wild-type oocyte with six DAPI-stained bodies and a *zim-1* mutant oocyte with eight DAPI-stained bodies are shown below.
(C-F) Images show pachytene-stage nuclei from samples that were hybridized with fluorescent DNA probes specific for different chromosomes. These and other experiments indicated that specific chromosomes consistently fail to synapse in each of the zim mutants.
(C) In *zim-1* mutants, probes to the left ends of chromosomes *II* (pink) and *III* (orange) are unpaired in the pachytene region.

(D) In *zim-2* mutants, a probe to the right end of chromosome *V* (yellow) is unpaired while a probe to the center of chromosome *IV* (red) is paired in the pachytene region (In this example, the *IV*C probe is a positive control).
(E,F) Probes to chromosomes *I* (E, green) and *IV* (F, red) are unpaired at pachytene in *zim-3* mutants.
Scale bars represent 5μm.

Figure 3. Pairing Of Chromosome *V* Is Not Stabilized In *zim-2* Mutants
(A) Diagram of a hermaphrodite gonad, indicating the five zones in which the pairing of FISH signals was scored.
(B) Genomic localizations of the three FISH probes used to quantify homolog pairing.
(C) The bar graphs indicate the fraction of paired FISH signals in each zone for wild-type (N2) and *zim-2(tm574)* hermaphrodites. Three probes were scored independently in the same samples: one from the left end of chromosome *V* (red), one from the right end of chromosome *V* (green), and one from the center of the *X* chromosome (blue). In *zim-2*, pairing of the chromosome *V* probes did not rise above the baseline levels observed in the premeiotic region (zone 1), whereas the *X* chromosome association rates and dynamics were very similar to what we observed in wild-type hermaphrodites.
(D) Numerical data corresponding to the graphs in (C) indicate the frequency of paired signals (and number of nuclei examined).

Figure 4. ZIM Proteins Localize To Discrete Foci At The Nuclear Envelope During Early Meiotic Prophase
(A) A wild-type gonad stained with DAPI and antibodies to ZIM-1 and ZIM-2. ZIM-1 and ZIM-2 foci localize predominantly to the transition zone of the gonad. Some foci can be seen outside of the transition zone/early pachytene region, but when overlaid with the DAPI, these foci are not nuclear and appear to be background.
(B,C) Near the end of the transition zone, most nuclei have two foci of ZIM-1 (green, B) and a single focus of ZIM-2 (red, C).
(D) In early pachytene, two foci of ZIM-3 (orange) can been visualized in each nucleus.
(E-G) Wild-type pachytene nuclei are stained with an antibody against nuclear lamin/LMN-1 (white) and ZIM-1 (E, green), ZIM-2 (F, red) and ZIM-3 (G, orange). All three ZIM antibodies appear inset in the nuclear envelope. These three images are single optical sections, which more clearly reveal the association between ZIM proteins and the lamina than 3D projections, but consequently not all ZIM-1 and ZIM-3 foci are visible in the plane.
Scale bars represent 5μm.

Figure 5. ZIM Proteins Localize To Autosomal Pairing Centers

(A-D) ZIM-1 colocalizes with FISH probes recognizing the left ends of chromosomes *II* and *III*. Two arrows point to the two ZIM-1 foci in a single nucleus and to the chromosomes *II* and *III* FISH probes which colocalize with them.
(E-H) ZIM-2 colocalizes with a FISH probe to the right end of chromosome *V*. An arrow points to a good example of this colocalization.
(I-P) ZIM-3 colocalizes with FISH probes to the right end of chromosome *I* (I-L) and the left end of chromosome *IV* (M-P). Arrows point to a ZIM-3 focus that colocalizes with the FISH probe in each example.
(A, E, I, and M) are DAPI-stained nuclei in late transition zone or early pachytene. (B, F, J, and N) are antibody staining against ZIM-1, ZIM-2, ZIM-3 and ZIM-3, respectively. (C, G, K, and O) are FISH probes to IIL, IIIL, VR, IR and IVL. (D, H, L, and P) are merges of the previous three images.
(Q) A schematic showing the correspondence of ZIM-1, -2, -3, and HIM-8 to the six *C. elegans* chromosomes. The region to which the PCs have been mapped genetically are demarcated in blue; the actual PCs are potentially much more restricted in size.
Scale bars represent 5μm.

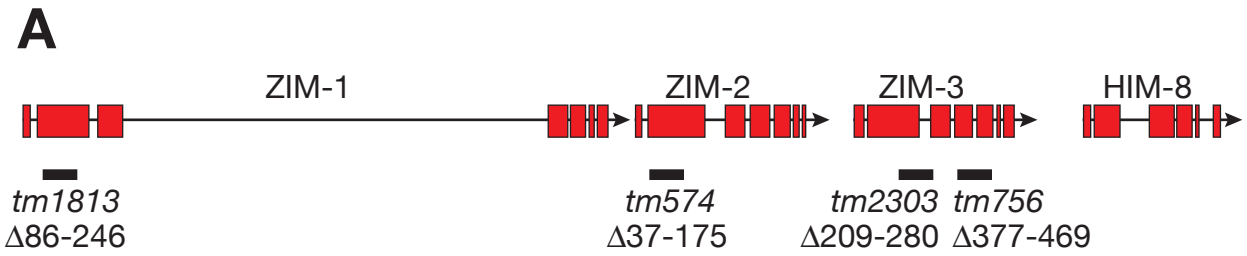
Figure 6. ZIM-1, ZIM-2, and ZIM-3 recognize distinct chromosomal foci and do not cluster in meiotic nuclei.
(A) Gonad from a *zim-2* hermaphrodite stained with DAPI and ZIM-1 shows an extended region of polarized nuclei (indicated by white line) and increased perdurance of ZIM-1 foci relative to wild-type (Figure 4A).
(B) DAPI and immunofluorescence of ZIM-1 (green) and ZIM-2 (red) [top three panels], and ZIM-3 (orange) and HIM-8 (yellow) [bottom three panels] in *zim-1, zim-2,* and *zim-3(tm756/tm2303)* meiotic nuclei. In all cases, the mutant protein is no longer detected, but the other three proteins show normal subcellular staining patterns.
(C) Transition zone nuclei stained with DAPI and antibodies against ZIM-1, ZIM-2, ZIM-3, and HIM-8 show several distinct foci at the nuclear periphery, indicating the absence of tight clustering among the different Pairing Centers.
Scale bars represent 5μm.

Figure 7. Evolution of the ZIM/HIM-8 Protein Family
(A) Schematic of the ZIM/HIM-8 operon in *C. elegans, C. briggsae,* and *C. remanei.* Predicted orthologs are linked by grey shading.
(B) ClustalX alignment of the Zn finger region of all ZIM/HIM-8 proteins from *C. elegans, C. briggsae,* and *C. remanei.* Red and yellow shading indicate identical and similar residues, respectively. Asterisks indicate zinc ion coordinating residues.
(C and D) Unrooted trees of the ZIM/HIM-8 proteins generated using ClustalX and TREEVIEW.

(C) The most conserved region of the proteins outside the zinc finger region was used to generate this tree. Colored blobs highlight branches of the tree that indicate greater conservation of this region among ZIM proteins within individual species than between species.
(D) A conserved C-terminal region containing the two C2H2 Zn fingers was used to generate this tree. Yellow blobs highlight branches of the tree that reveal orthologous relationships among ZIM proteins among three nematode species.

Figure S1. Validation of Cb-HIM-8 function
(A-B) Six DAPI-stained bodies are detected at diakinesis in wild-type *C. remanei* and *C.briggsae* oocytes.
(C) Following *Cb-him-8* RNAi, seven DAPI-stained bodies are visible at diakinesis, including two univalents indicated by yellow arrows.
(D) Percent males and mean number of DAPI-stained bodies were scored in *C. briggsae* wild-type, *him-8*^{RNAi}, and *him-8*^{RNAi} hermaphrodites.
(E) The percent males in *C. briggsae him-8*^{RNAi} hermaphrodites with the most severe phenotype are indicated.
(F) A graph indicating the number of nuclei with a given number of DAPI-stained bodies at diakinesis in *C. briggsae* wild-type, *him-8*^{RNAi}, and *him-8*^{RNAi} hermaphrodites.
Scale bars represent 5μm.

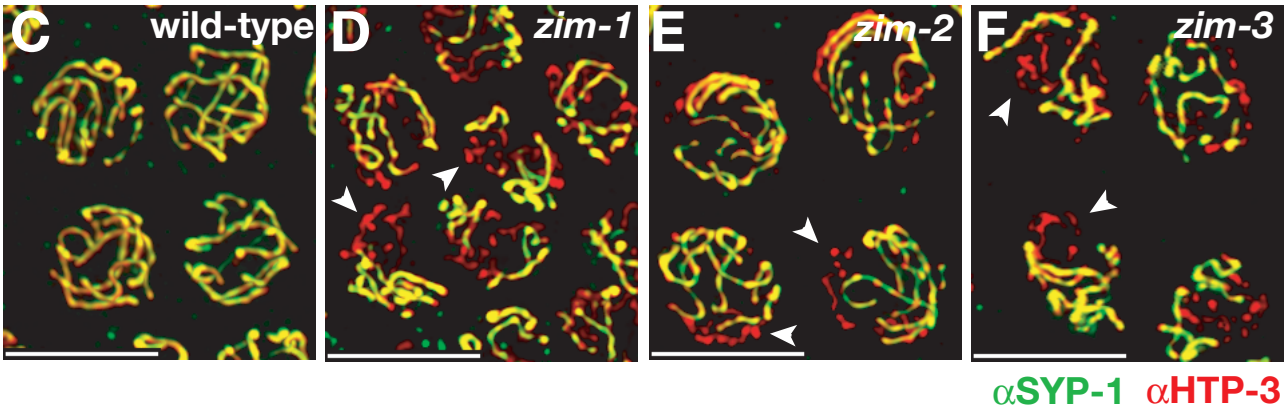
Figure
Click here to download Figure: Figure1.pdf



B

genotype	% males (# of adults scored)	% viable embryos (# of embryos scored)
wild-type[a]	0.1 (1954)	100.0 (372)
zim-1(tm1813)	6.7 (491)	26.1 (1879)
zim-2(tm574)	1.3 (695)	68.4 (1479)
zim-3(tm2303)	6.7 (405)	23.1 (1750)

a. data from Phillips et al. (2005)



Figure

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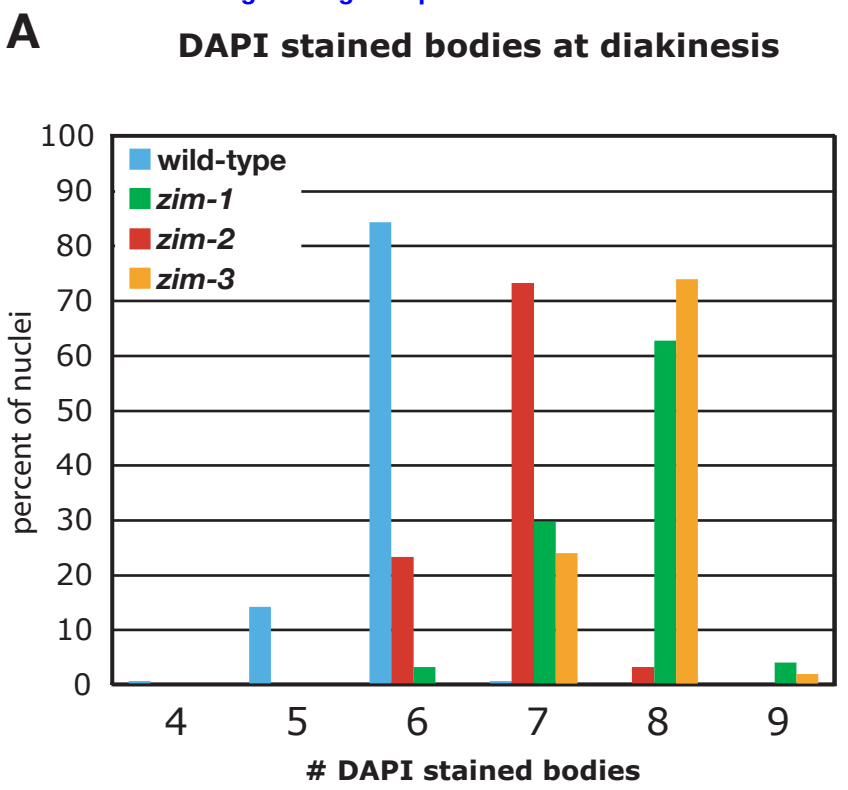
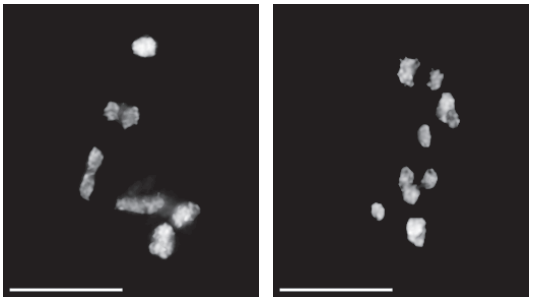
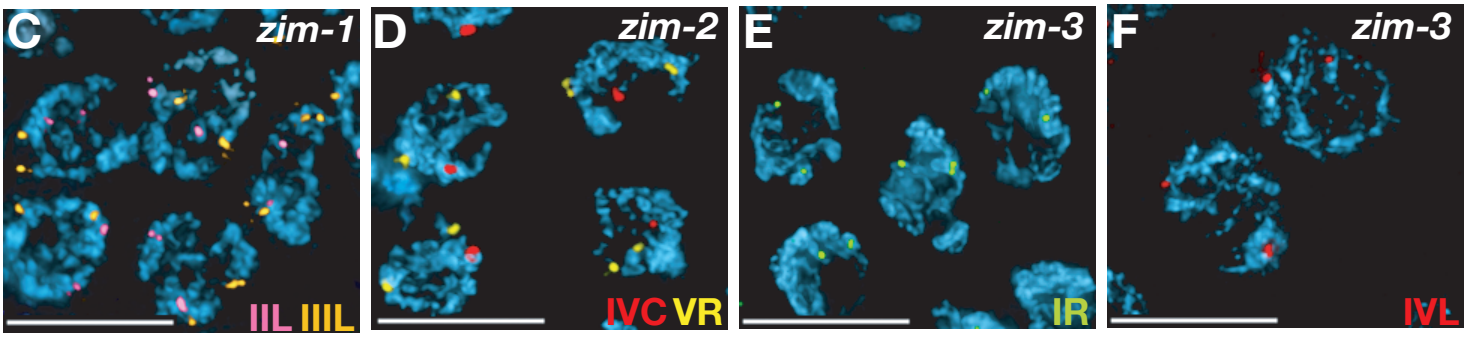


B

genotype	average # DAPI stained bodies (# of nuclei scored)
wild-type	5.9 (141)
zim-1	7.7 (121)
zim-2	6.8 (90)
zim-3	7.8 (100)

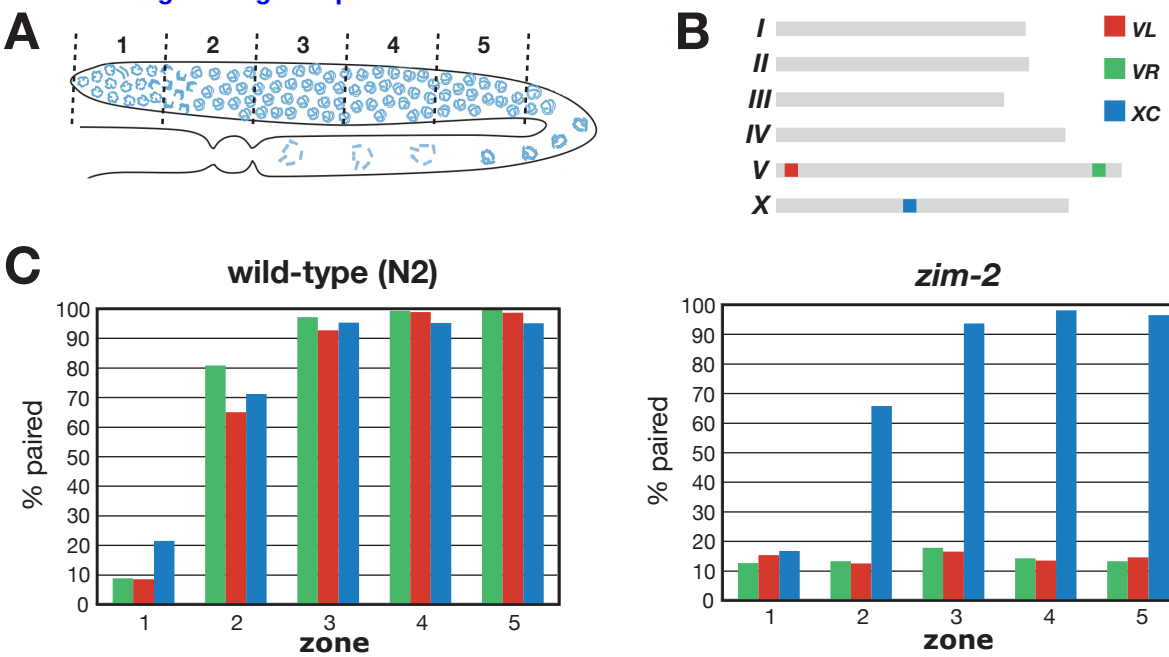




Phillips and Dernburg
Figure 2

Figure

Click here to download Figure: Figure3.pdf



D

VR	1	2	3	4	5
wild-type (N2)	8.9 (349)	80.9 (424)	97.2 (498)	99.3 (443)	100 (305)
***zim-2* (*tm574*)**	12.7 (292)	13.3 (278)	17.9 (302)	14.3 (280)	13.4 (232)

VL	1	2	3	4	5
wild-type (N2)	8.6 (349)	64.9 (424)	92.8 (498)	98.9 (443)	98.7 (305)
***zim-2* (*tm574*)**	15.4 (292)	12.6 (278)	16.6 (302)	13.6 (280)	14.7 (232)

XC	1	2	3	4	5
wild-type (N2)	21.5 (349)	71.2 (424)	95.4 (498)	95.3 (443)	95.1 (305)
***zim-2* (*tm574*)**	16.8 (292)	65.8 (278)	93.7 (302)	98.2 (280)	96.6 (232)

Figure

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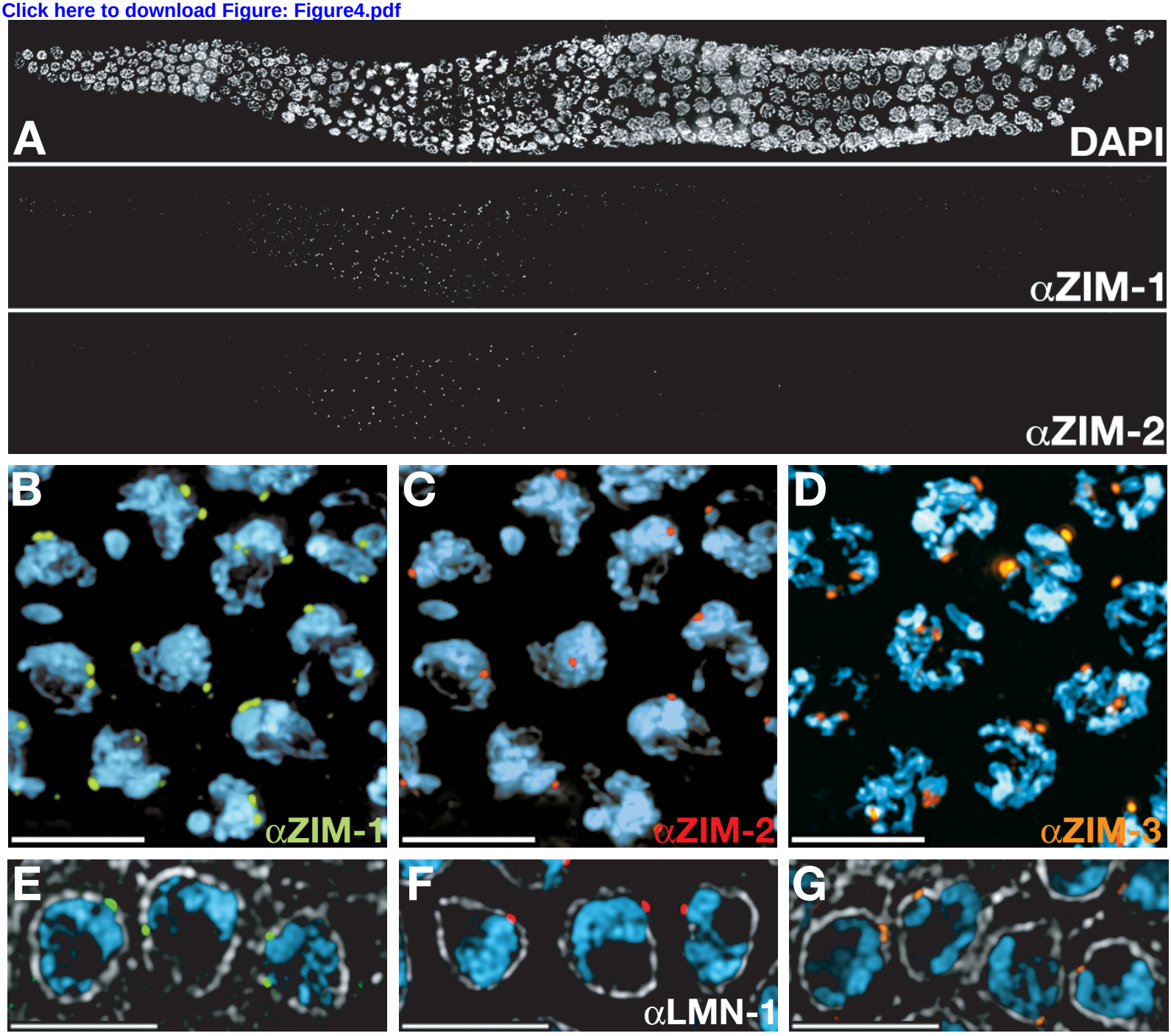

Phillips and Dernburg
Figure 4

Figure
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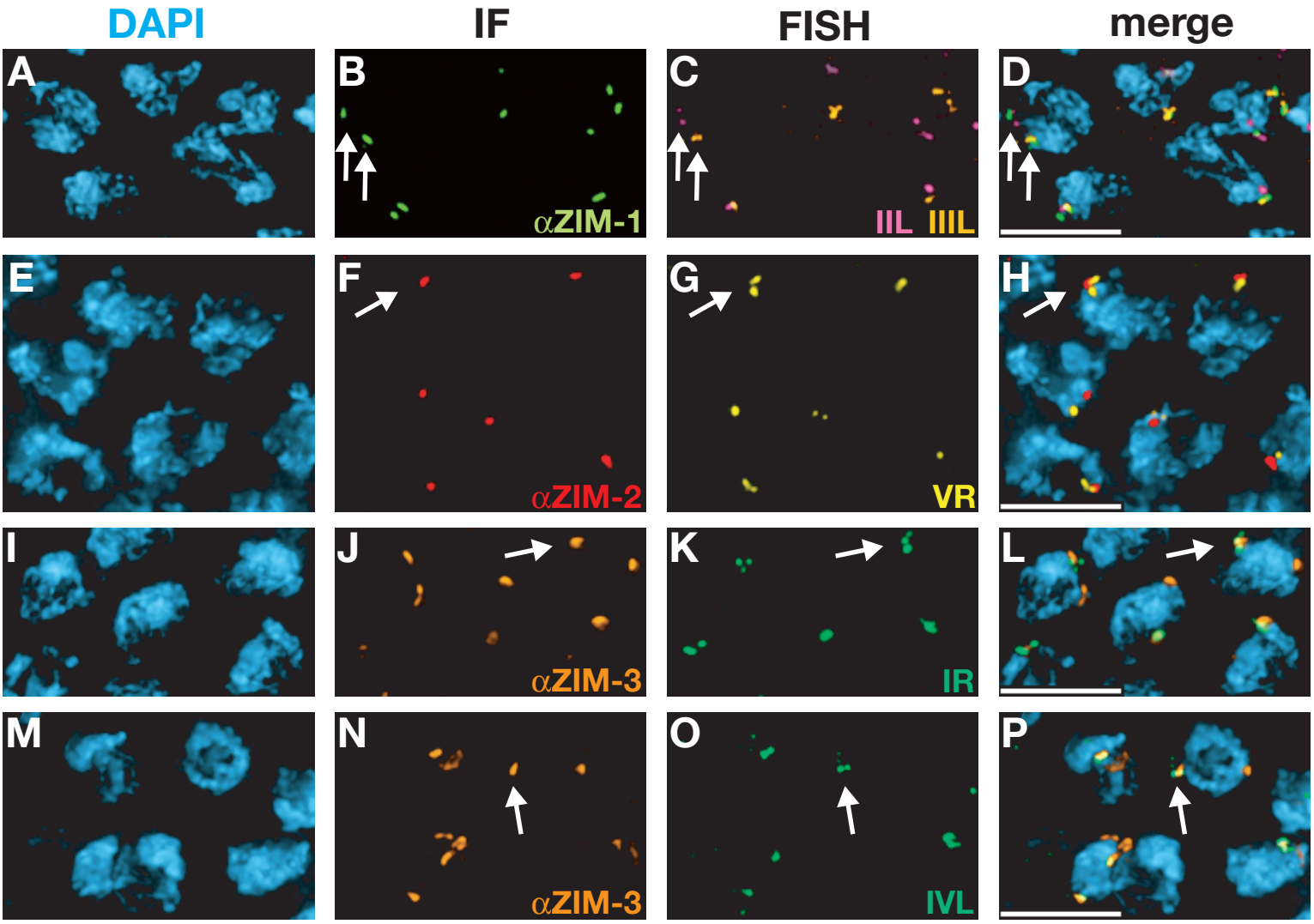

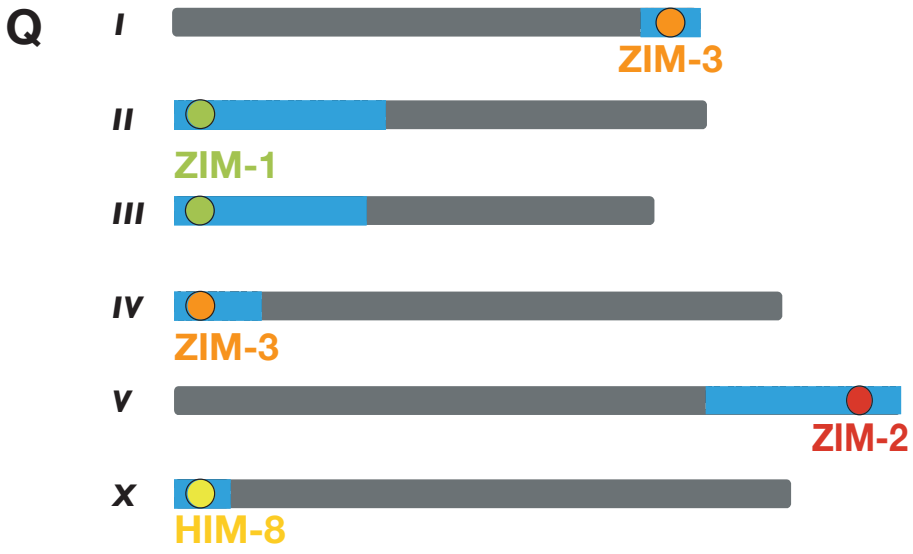

Phillips and Dernburg
Figure 5

Figure
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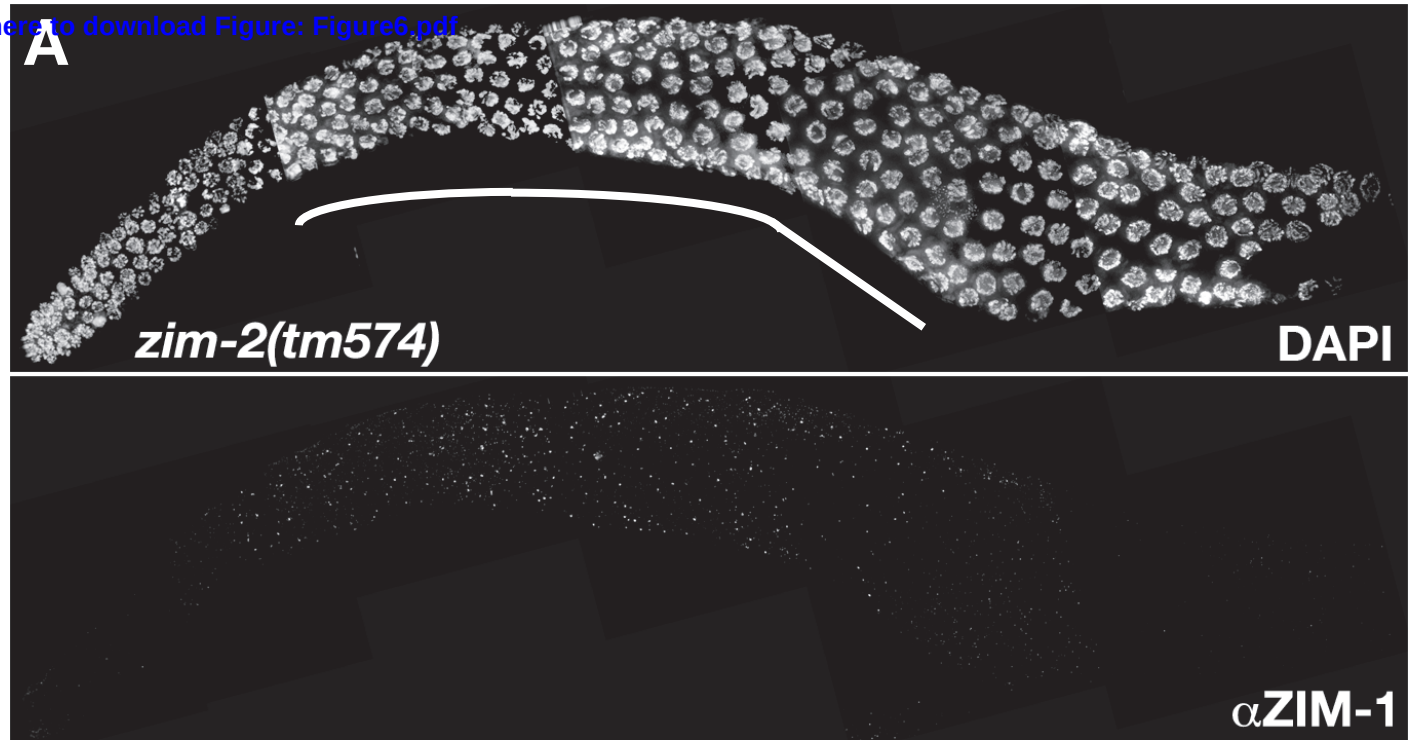

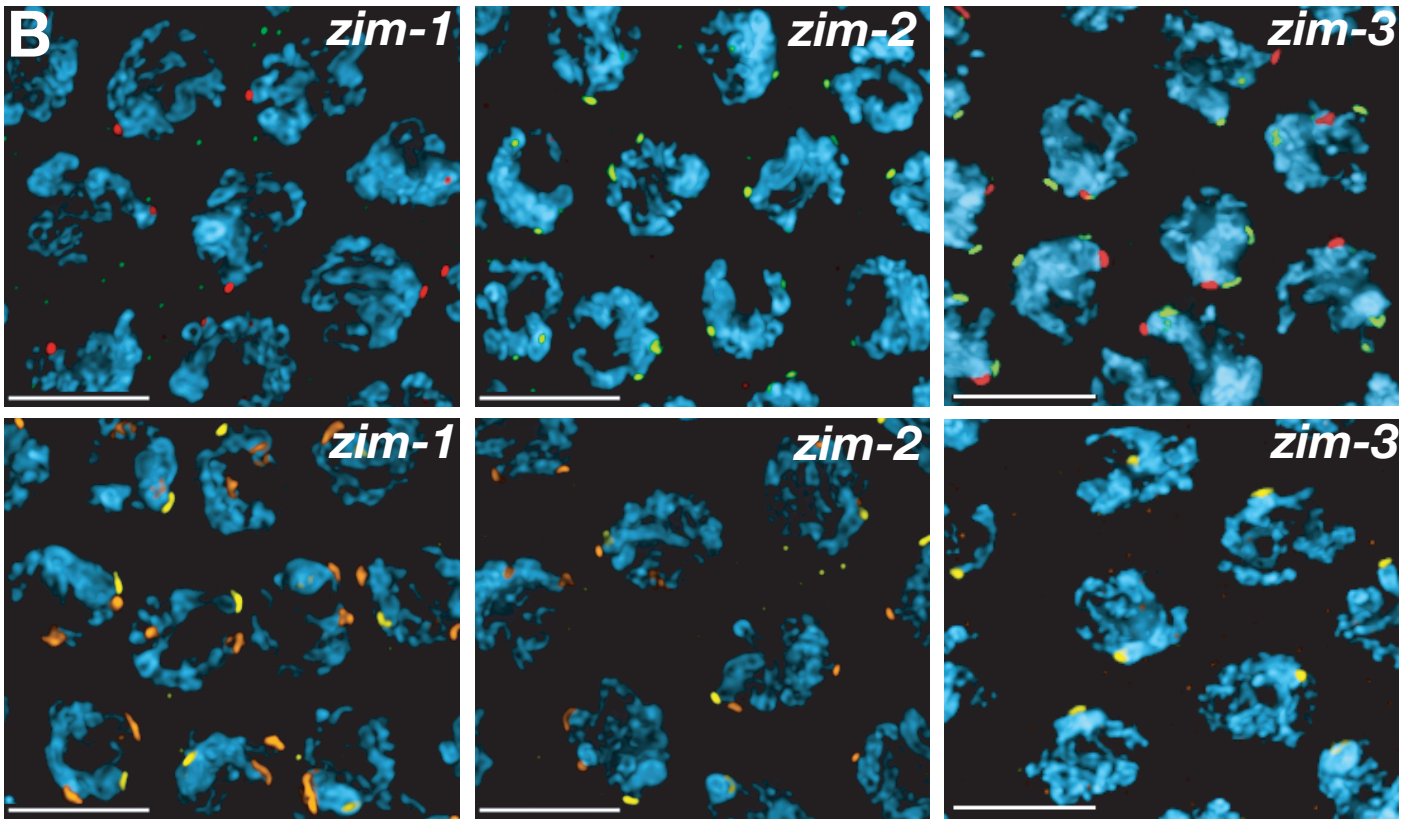

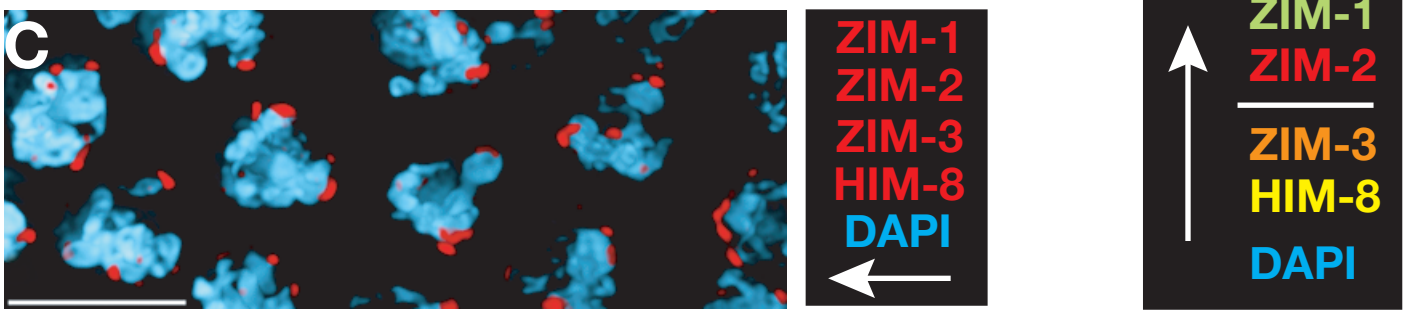

Phillips and Dernburg
Figure 6

Figure

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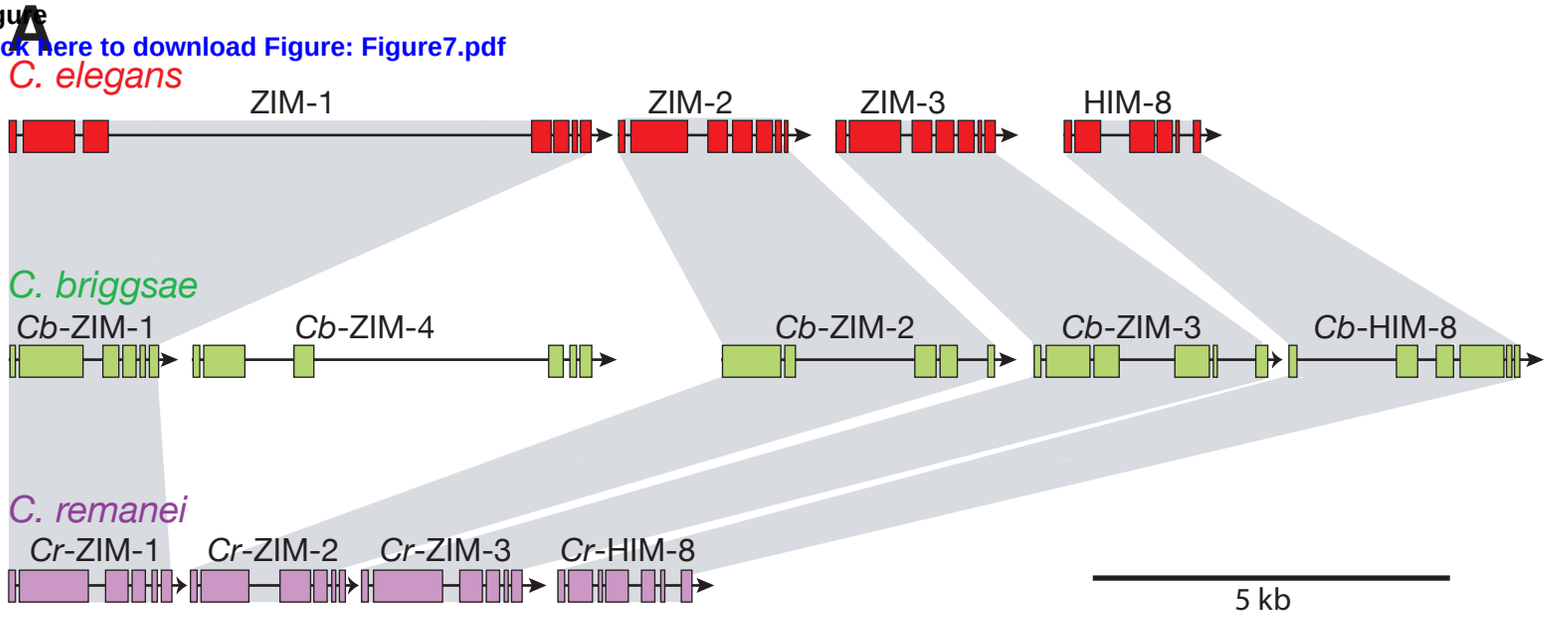

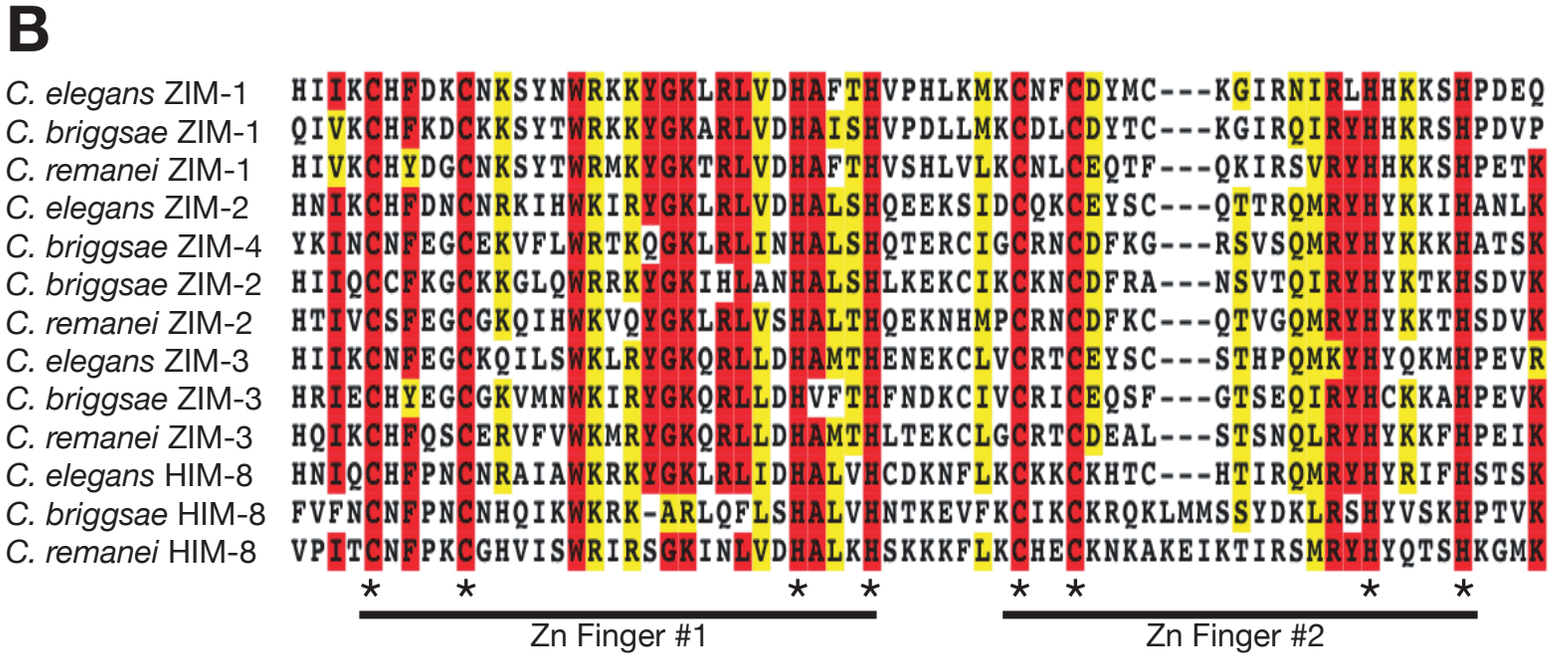

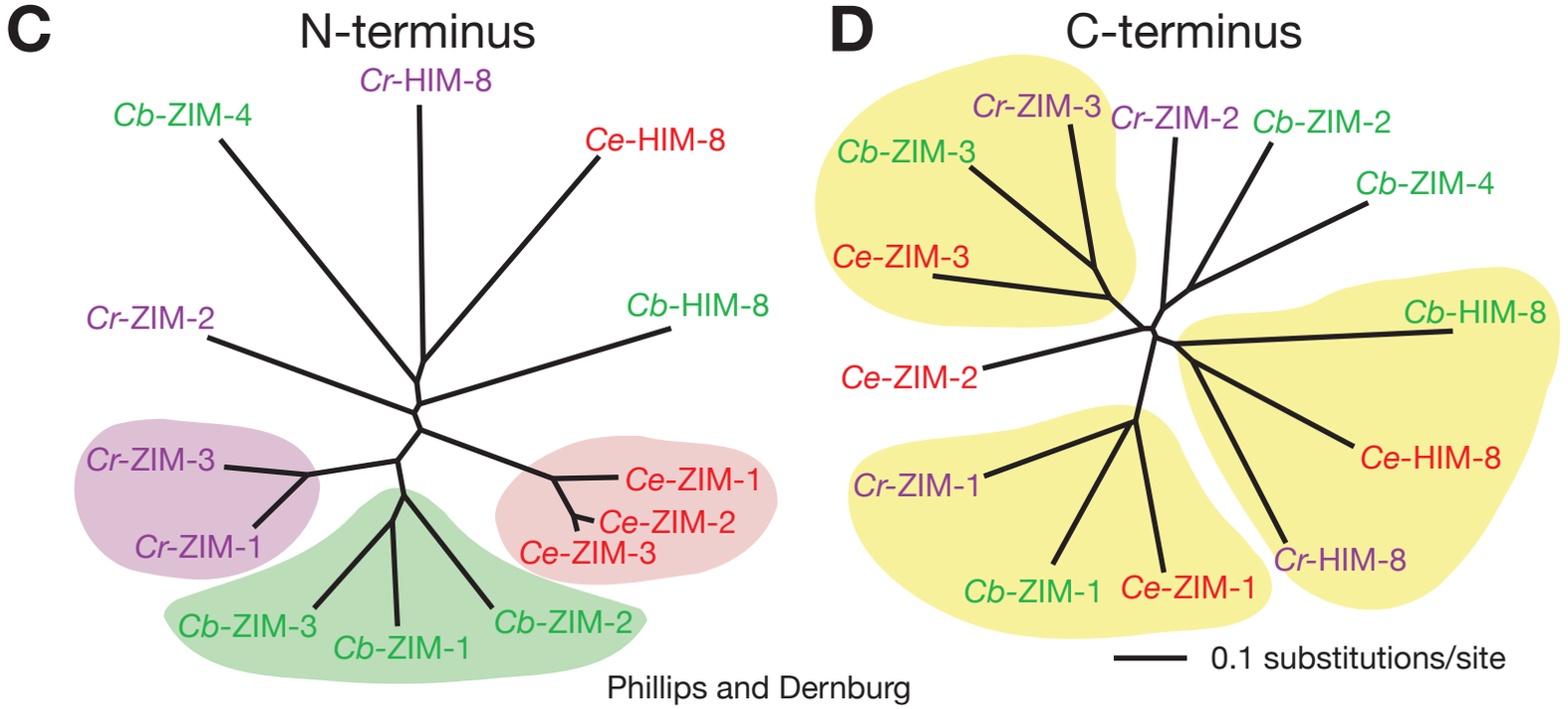

Phillips and Dernburg

Figure 7

Supplemental Data – Phillips and Dernburg
C. remanei genomic and cDNA sequences

Cr-genomic sequence Contigs6.71-6.74 (gaps filled by our sequencing in red)

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Cr-zim-1 cDNA
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Cr-zim-2 cDNA
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Cr-zim-3 cDNA

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Cr-him-8 cDNA

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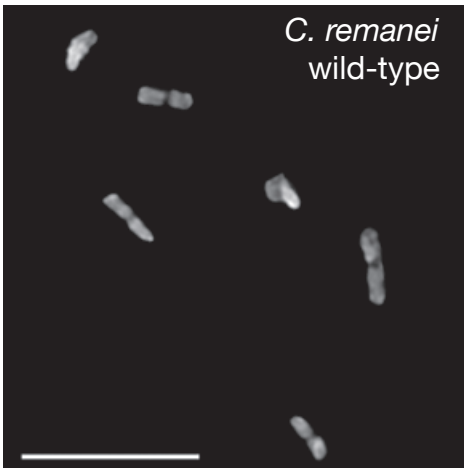
A



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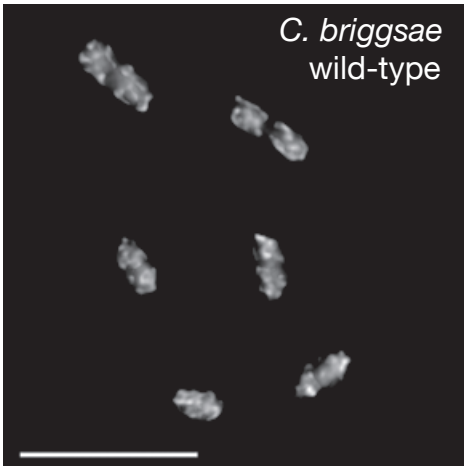

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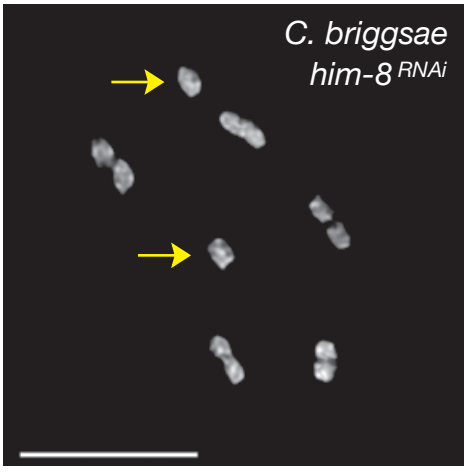
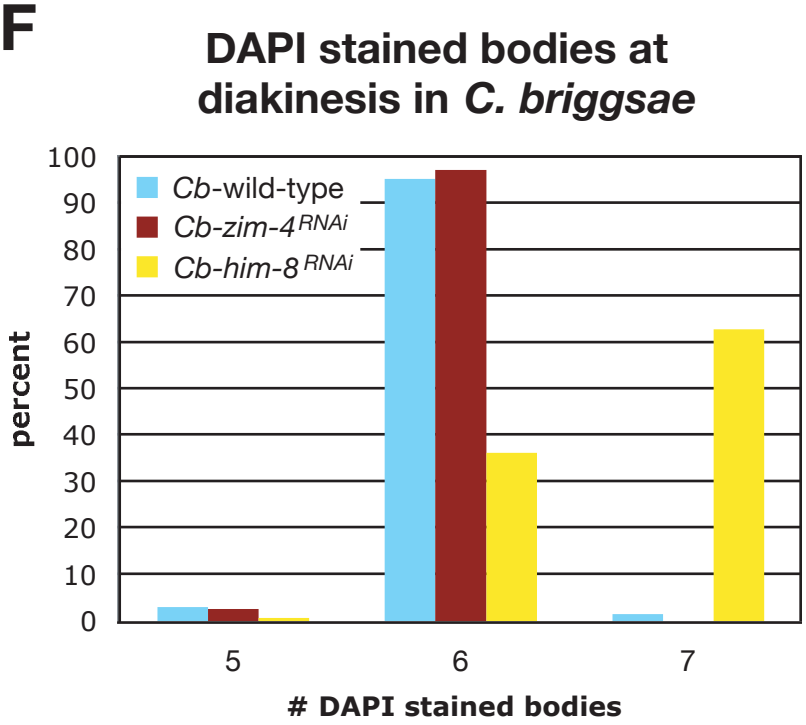


E

% males on *Cb-him-8* RNAi plates with highest number of males (total number of adults on plate)
31.1 (132)
29.2 (195)
28.9 (114)
25.8 (62)
22.1 (104)

F



D

genotype	% males (total number of adults scored)	mean number of DAPI stained bodies (total number of nuclei scored)
Cb-wild-type	0.1 (1818)	6.0 (66)
Cb-zim-4 RNAi	0.2 (4061)	6.0 (117)
Cb-him-8 RNAi	9.0 (6299)	6.6 (146)

Phillips and Dernburg
Supplementary Figure 1