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Identification of Chromosome Sequence Motifs That Mediate Meiotic Pairing and Synapsis in *C. elegans*

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Abstract

C. elegans chromosomes contain specialized regions called pairing centers (PCs) that mediate homologous pairing and synapsis during meiosis. Four related proteins, ZIM-1, -2, -3, and HIM-8, associate with these sites and are required for their essential functions. Here we show that short sequence elements enriched in the corresponding chromosome regions selectively recruit these proteins *in vivo*. *In vitro* analysis using SELEX indicates that each protein's binding specificity arises from a combination of two zinc fingers and an adjacent domain. Insertion of a cluster of recruiting motifs into a chromosome lacking its endogenous PC is sufficient to restore homologous pairing, synapsis, crossover recombination, and segregation. These findings help to illuminate how chromosome sites mediate essential aspects of meiotic chromosome dynamics.

Studies of genome rearrangements in the nematode *Caenorhabditis elegans* have shown that particular regions near one end of each chromosome are required in *cis* for homologous recombination and segregation during meiosis. Translocations or deletions of these regions suppress genetic exchange across large chromosome regions[1-5]. These "pairing centers" (PCs) stabilize pairing and promote the assembly of the synaptonemal complex (SC) between homologous chromosomes[6, 7].

A family of four paralogous proteins, each containing two motifs resembling C2H2 zinc fingers (ZnFs), is required for PC function[8, 9]. Each protein localizes to the PCs of one or two pairs of chromosomes during early meiotic prophase: ZIM-1 on chromosomes *II* and *III*, ZIM-2 on *V*, ZIM-3 on *I* and *IV*, and HIM-8 on the *X* chromosome. Loss of any of these proteins results in defects in pairing, synapsis, recombination, and segregation of the corresponding chromosomes.

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cis-acting elements that underlie PC function have not yet been described. Here we identify sequence motifs enriched on each chromosome that specifically recruit the cognate ZnF protein required for that chromosome to undergo faithful meiotic segregation. Our identification of sites that recruit each protein *in vivo* is corroborated by *in vitro* binding experiments that illuminate the basis for their sequence specificity. Integration of these sequences onto a chromosome deficient for PC activity is sufficient to restore meiotic chromosome pairing and synapsis. Moreover, we demonstrate that these recruitment motifs do not require a specific chromosome position, and that one ZnF protein can substitute for another to promote meiotic chromosome interactions.

Results and Discussion

Identification of *X* Chromosome Pairing Center sequences

The *X* chromosome PC has been previously mapped to the region distal to (or left of, by *C. elegans* convention) the *dpy-3* locus, 2.15 Mb from the left telomere[3, 10]. *X* chromosomes lacking this region usually fail to synapse or undergo exchange and consequently missegregate, resulting in an elevated frequency of *XO* (male) progeny[3], known as the High incidence of males, or Him, phenotype[11].

To delimit the region containing the *X* chromosome PC more precisely, deficiencies were mapped using single nucleotide polymorphisms (SNPs)[12, 13]. We analyzed three deficiencies that result in the loss of HIM-8 localization from the *X* chromosome and remove all PC function (Fig. 1b-d) [3]. Each of these three deficiencies lacked all markers tested between 50 Kb and 1.46 Mb from the left end (see Methods; Fig. 1a), but did not delete a marker at 2.07 Mb. In contrast, *yDf19*, an *X* chromosome deficiency that retains HIM-8 staining (Fig. 1e) and undergoes normal meiotic segregation[14] lacked the leftmost markers scored, but its right breakpoint was found to lie between 1.06 and 1.17 Mb from the left end (Fig. 1a). These data indicate that elements sufficient to recruit HIM-8 and confer PC activity are contained within sequences between 1.06 and 2.07 Mb from the left end of the *X* chromosome.

Candidate sequences within this 1 Mb region were injected into wild-type *C. elegans* to test for HIM-8 binding. The resulting transgenic animals carried high copy extrachromosomal arrays, which typically contain megabases of the injected DNA and are transmitted through mitosis and meiosis (see Methods)[15]. Combining FISH with immunofluorescence, we tested whether candidate arrays could recruit HIM-8 in germline nuclei. Although this approach is unbiased with respect to candidate sequences, it does require that HIM-8 recognize a sequence motif or other element within the chromatin context of an extrachromosomal array, which undergoes transcriptional silencing and enriched H3K9 dimethylation in germline nuclei[16]. While we did not know *a priori* whether HIM-8 would bind to arrays, we were encouraged by the success of an analogous approach to identify sequence elements that recruit *C. elegans* dosage compensation complex proteins in somatic nuclei[17, 18].

From an initial pool of cosmids that recruited HIM-8, we narrowed the recruitment activity to smaller fragments, ultimately to a 539 bp amplicon (Fig. 1f; Supplementary Information,

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Table S1). Centered within this short sequence are five and a half copies of a 21 bp repeat, and no other repetitive element, coding sequence, or other feature of obvious interest.

Computational analysis revealed that a 12 bp motif, (TTGGTCAGTGCT) contained within the larger repeat is enriched on the *X* chromosome relative to the autosomes and in the PC region relative to the entire *X* chromosome (Supplementary Information, Fig. S1a). When degeneracy was allowed, we found that some closely related sequences were also enriched in the PC region, and that TTGGTCAGTGCA, which differs at the 3' nucleotide, is even more abundant than the original motif (Fig. 2; Supplementary Information, Fig. S1). Interestingly, a version of this sequence lacking the 5' base was previously identified via computational methods as the most overrepresented oligonucleotide on the *X* chromosome and named CeRep50[19].

To test whether TTGGTCAGTGCA could also recruit HIM-8, we amplified two short regions from the left end of the *X* chromosome, each containing several copies of this motif with different flanking sequences. Both amplicons recruited HIM-8 (Fig. 1g; Supplementary Information, Table S1), as did a synthetic oligonucleotide containing four tandem copies of this motif interspersed with flanking spacers. TTGGTCAGTGCA is therefore sufficient to recruit HIM-8.

Both of these related HIM-8 recruiting motifs are highly enriched near the left end of the *X* chromosome and are most often found in short, tandemly oriented clusters with a predominant 21-base periodicity (Supplementary Information, Table S2)[19]. Many copies lie within introns of known or predicted genes. Small clusters or isolated occurrences of these motifs can also be found elsewhere on the *X* chromosome and occasionally on autosomes. *yDf19*, the deficiency chromosome with a functional PC, retains about half of the motifs normally found in the left 2 Mb of the *X* chromosome, while *meDf2*, *meDf3*, and *meDf5* remove between 94-100% of the motifs found in this dense cluster.

Immunofluorescent detection of HIM-8 in germline nuclei reveals a single primary association site in the genome, which corresponds to the region of the *X* chromosome removed by *meDf2*, *meDf3*, and *meDf5* (Fig. 1b-d)[9]. However, granular staining of HIM-8 is detected elsewhere on the chromatin, which may reflect binding to related motifs elsewhere in the genome. HIM-8 may weakly promote *X* chromosome segregation even in the absence of the PC, since loss of *him-8* function results in a more severe meiotic phenotype than *X* chromosome PC deficiencies[9]. To assay the recruitment potential of clusters outside the PC, we amplified clusters of motifs from the center and right end of the *X* chromosome, and also from Chromosome *III*. Each of these amplicons recruits HIM-8 in the high-copy extrachromosomal array assay (Fig. 1h; Supplementary Information, Table S1). While HIM-8 recruitment motifs elsewhere on the *X* may contribute to segregation, they lack key PC functions; in particular, they do not measurably stabilize homolog pairing in the absence of synapsis[7]. This suggests that PCs require a minimal density of binding sites, or perhaps other *cis*-acting components, for full function.

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Identification of autosomal pairing center sequence motifs

Our evidence indicates that HIM-8 is recruited *in vivo* by a sequence previously identified *in silico* as the most overrepresented short oligonucleotide on the *X* chromosome. Prior computational analysis also identified short sequences that are most overrepresented on each autosome relative to the other five chromosomes. Each of these sequences, designated as CeRep45-50, was found to be asymmetrically enriched near one end of a single chromosome[19]. These regions of enrichment roughly corresponded with PCs, which have been mapped to varying precision on different chromosomes. However, these observations were based entirely on computational analysis, and no functions have previously been demonstrated for these abundant motifs.

We revisited and modified the previous analysis in light of the knowledge that PCs on Chromosomes *I* and *IV* share a common ZnF protein (ZIM-3), as do *II* and *III* (ZIM-1)[8]. We found that CeRep45, the most overrepresented sequence on Chromosome *I*[19], is also highly enriched within a 120-kb window within the PC region on *IV* (Fig. 2; Supplementary Information, Fig. S2). Interestingly, while most repeats on *I* are clustered in alternating orientation (or “inverted” clusters, Supplementary Information, Table S2) with a total period of 68 bp, the copies on *IV* are mostly in tandemly oriented clusters with a 19 bp periodicity (Supplementary Information, Table S2). Amplicons spanning clusters of this motif from either Chromosome *I* or *IV* strongly recruited ZIM-3 to extrachromosomal arrays (Supplementary Information, Fig. S5a, S5c, Table S1).

Chromosomes *II* and *III* both require ZIM-1 for meiotic pairing and synapsis[8]. While neither CeRep45 nor CeRep46, the sequences most overrepresented on Chromosomes *II* and *III*, respectively, is highly abundant on the other chromosome, we found that both repeats are associated with a distinct motif, $T^{G}/_{T}$GGTCTGCTA, which is enriched on both chromosomes (Fig. 2 Supplementary Information, Fig. S3). Amplicons containing clusters of these elements showed specific recruitment of ZIM-1 (Supplementary Information, Fig. S5b, Table S1). While this motif is predominantly in inverted clusters on both *II* and *III*, the predominant spacing of these elements is different on the two chromosomes (Supplementary Information, Table S2).

Recruitment of PC proteins to *V* was more enigmatic. The CeRep49 sequence identified by Sanford and Perry ([T]TGGGCGCTGCT) seemed an excellent candidate for ZIM-2 recruitment, in that it is highly enriched on Chromosome *V* and also resembles in base composition and length the motifs that recruit the other proteins (Fig. 2; Supplementary Information, Fig. S4). Indeed, we did find that one cluster of this repeat specifically recruited ZIM-2, although somewhat less robustly than other ZIM-recruiting arrays (Supplementary Information, Fig. S5d, Table S1). However, a different cluster from Chromosome *V* containing both this motif and the motif TTGGTCGCTGCT, which differs at the underlined base, strongly recruited both ZIM-2 and HIM-8 (Supplementary Information, Fig. S5e-f, Table S1). As shown previously[19], the organization of CeRep49 repeats on Chromosome *V* is bimodal with respect to spacing, with prominent 18- and 32-base periodicities. Clusters with 32 bp spacing contain CeRep49 alone, while clusters with 18 bp spacing always include this G–>T variant motif (Supplementary Information, Table

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S2). It is unclear whether the co-recruitment of HIM-8 to arrays containing the second class of cluster is due to the variant sequence, or to the distinct spacing, but it is suggestive that the T variant is more similar to the *X* chromosome repeat, TTGGTCAGTGCA. No obvious recruitment of HIM-8 is detected cytologically on Chromosome *V*, and genetic evidence indicates that only ZIM-2 is required for efficient pairing and synapsis of this chromosome[8]. Nevertheless, indirect evidence suggests that HIM-8 might contribute to pairing of Chromosome *V* in the absence of ZIM-2, since appreciable crossing-over is detected cytologically in a *zim-2* mutant[8].

In *vitro* analysis of Pairing Center sequences

HIM-8 and the ZIM proteins each contain two short domains resembling C2H2 ZnFs[20], the most common DNA binding motif in metazoa[21]. Data presented here and in previous work[8, 9] indicated that defined chromosomal sequences are able to recruit HIM-8 and the ZIM proteins *in vivo*, and that missense mutations in the ZnF domains of HIM-8 disrupt chromosome association[9]. However, the spacing between the Zn-coordinating Cys and His residues in the first finger of the ZIM/HIM-8 family is distinct from the canonical C2H2 spacing seen in proteins such as Zif268[22] or Sp1[23]. Furthermore, a single canonical C2H2 ZnF usually specifies only a 3 or 4 bp subsite[22], raising the question of how these unusual two-ZnF proteins might recognize the ~12 bp non-palindromic sequences we identified.

To determine whether the C2H2 ZnFs in the ZIM/HIM-8 proteins can bind DNA sequence-specifically, we used a SELEX assay, as previously described[24]. Briefly, protein fragments expressed *in vitro* were incubated with a library of double-stranded DNA fragments carrying a randomized 21 bp stretch of DNA. The protein-bound DNA was isolated and amplified, and the cycle was reiterated three more times, after which the bound DNA fragments were sequenced and analyzed to derive a consensus. Somewhat surprisingly, the assay produced very similar results when this analysis was performed with the core ZnF domains of HIM-8, ZIM-2, and ZIM-3. In all three cases, the pentamer motif “TTGGC” clearly emerged as the preferred binding site (Fig. 3; Supplementary Information, Table S3). Interestingly, this sequence is similar but not identical to one end of all of the binding motifs we identified *in vivo*. We next expressed a longer fragment of HIM-8, including the region from the ZnF domain to the C-terminus of the protein. As shown (Fig. 3b; Supplementary Information, Table S3), this fragment bound specifically to oligonucleotides containing the consensus $^{T}/_{A}$TGGTCAGTGC, which is identical to the full length HIM-8 recruiting repeat we identified *in vivo*.

Taken together, the SELEX results indicate that HIM-8 uses a composite protein-DNA interaction domain to recognize its full target site. We infer that the distinct recruitment motifs of the ZIM proteins are likely recognized by the combined specificities of their ZnF domain and a short adjacent C-terminal domain in each protein, and that the Zn fingers likely specify one end of each binding sites containing “TTGG.” These findings suggest a direct correspondence between each ZnF protein and the sequences we identified *in vivo*, implying that no other cofactors are likely required to recruit these proteins to their cognate chromosome sequences. Future work may elucidate how these novel composite DNA-binding domains interact with their cognate binding sites.

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ZIM/HIM-8 recruiting arrays interact with the nuclear envelope

Chromosomal PCs are physically associated with the nuclear envelope during early meiotic prophase[8, 9]. We noticed that when ZIM/HIM-8 proteins are recruited to the extrachromosomal arrays, they concentrate at the interface between the array and the nuclear envelope (see Fig. 1f-h). This suggests that the ZnF proteins recruit their binding sites to the periphery of the array and the nucleus. In work detailed elsewhere (A. Sato, CMP, AFD; Manuscript in preparation), we have found that the sites of contact between endogenous PCs and the nuclear envelope are enriched for several proteins that contribute to chromosome segregation. These include the inner and outer nuclear membrane proteins SUN-1 and ZYG-12, which concentrate at discrete patches along the nuclear surface during early prophase[25, 26]. We therefore tested whether arrays that recruit ZIM/HIM-8 proteins also associate with these nuclear envelope components. We compared the behavior of three extrachromosomal arrays, two that recruit different ZnF proteins and a control, non-recruiting array (Fig. 1g; Supplementary Information, Fig. S5c, Table S1). The ZIM/HIM-8 recruiting arrays clearly associated with patches of SUN-1 and ZYG-12 at the nuclear envelope, in contrast to the non-recruiting array (Fig. 4; data not shown). Thus, ZIM/HIM-8 recruitment motifs are sufficient to link the arrays to a protein complex that likely tethers these sequences to the nuclear envelope and mediates interactions with cytoplasmic dynein and microtubules (A. Sato, CMP, AFD; Manuscript in preparation).

HIM-8 recruiting sequences are sufficient to restore PC activity to the *X* Chromosome

PCs contribute to meiotic chromosomal segregation by both stabilizing homolog pairing and promoting synapsis[7]. To test whether a dense cluster of HIM-8 recruitment motifs is sufficient to restore these PC functions to an *X* chromosome lacking its endogenous PC, a HIM-8 recruiting extrachromosomal array was integrated onto the PC-deficient *meDf2* chromosome (*meDf2 ieIs5*; see Methods; Fig. 5a). Whereas the *X* chromosomes in *meDf2* homozygotes usually fail to synapse (Fig. 5b)[7], in *meDf2 ieIs5* nearly all nuclei showed complete homologous synapsis (Fig. 5c), demonstrating that HIM-8 recruitment by the inserted array can mediate proper synapsis of the X chromosomes. We also analyzed pairing of the *X* chromosomes in the absence of synapsis by knocking down the expression of *syp-2*, an essential SC component[27], by RNA interference (RNAi). Throughout the early meiotic region of the gonad, hybridization to the integrated array showed only a single region of staining (Fig. 5d), indicating that these inserted sequences can stabilize pairing between homologous *X* chromosomes in the absence of synapsis. By introducing the *him-8 (tm611)* null allele[9], we found that both the pairing and synapsis mediated by the integrated array require HIM-8 function (Fig. 5e and data not shown), much as these events do when mediated by the endogenous *X* chromosome PC.

Pairing and synapsis enable chromosomes to complete crossover recombination, which is required for chiasma formation and homolog segregation at the first meiotic division. To determine whether *meDf2 ieIs5* chromosomes undergo crossing-over, we scored the frequency of bivalents at diakinesis (Fig. 5f-h). As shown previously[3], most oocytes (65.6%) in *meDf2* hermaphrodites displayed univalent *X* chromosomes. By contrast, 99.4% of oocyte nuclei in *meDf2 ieIs5* homozygotes exhibited bivalent (recombinant) *X* chromosomes. The fidelity of *X* chromosome meiotic segregation can be quantified in *C. elegans* by the

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frequency of male self-progeny produced by hermaphrodites. *meDf2 ieIs5* hermaphrodites produced 1.8% males, dramatically fewer than *meDf2* homozygotes (33.1%) (Fig. 5i). We conclude that the integration of many copies of a 539 bp segment that recruits HIM-8 rescues pairing, synapsis, crossing-over, and segregation defects arising from deletion of the endogenous PC.

PC function does not require a specific chromosome position or ZnF protein

In our initial rescue experiment, HIM-8 motifs were fortuitously integrated towards the left end of the *meDf2* chromosome, near their natural location. We carried out additional experiments to ask whether the position on the chromosome is critical for PC activity, and also whether a particular chromosome requires a specific member of the ZIM/HIM-8 family. Identification of sequences that recruit both HIM-8 and ZIM-2 to arrays allowed us to address these questions about PC plasticity. We UV-irradiated animals carrying such an array and screened for integration events onto either Chromosome *V* or *X* (Supplementary Information, Fig. S5e-f). Two independent integrations into the left (non-PC) arm of chromosome *V* were recovered (*ieIs12* and *ieIs13*), as were two integrations on the right (non-PC) arm of *X* (*ieIs14* and *ieIs15*). The integrated arrays on Chromosome *V* were homozygosed and crossed to *zim-2 (tm574)*, which eliminates ZIM-2 activity[8], while the *X* chromosome integrants were crossed to *him-8 (mn253)* to abrogate HIM-8 function. In all cases the presence of the array on Chromosome *V* or *X* resulted in dramatic restoration of bivalent formation relative to *zim-2* or *him-8* mutants, respectively (Fig. 6a-e). Disjunction of the *X* chromosome (as measured by the frequency of male self-progeny) was also rescued in the absence of HIM-8, presumably by ZIM-2 association with the *X*-linked integrated array (Fig. 6f). These results indicate that ZIM-2 can promote crossing-over on the *X* chromosome, that HIM-8 can promote crossing-over on *V*, and that the chromosomal position of the major ZIM/HIM-8 binding cluster is plastic.

Integration of the same extrachromosomal array onto two different chromosomes also provided an opportunity to address whether such “artificial PCs” can promote pairing and synapsis between non-homologous chromosomes. We generated trans-heterozygotes by crossing worms with the ZIM-2/HIM-8 recruiting array integrated on *V* (*ieIs12* or *ieIs13*) to animals with the same array integrated on the *X* chromosomes (*ieIs14* or *ieIs15*). We looked at whether such “matching” PCs could stabilize pairing between non-homologous chromosomes in the absence of synapsis, and/or promote non-homologous synapsis. To look at pairing in the absence of synapsis, we visualized the integrated arrays in worms subjected to *syp-2* RNAi. In all four *trans*-heterozygous combinations, the ZIM-2/HIM-8 recruiting arrays on *X* and *V* were consistently paired (99% of nuclei) throughout the normal leptotene/zygotene and pachytene regions of the gonad (Fig. 6g-h; Supplementary Information, Table S4), indicating that non-homologous chromosomes did indeed undergo robust, stable pairing.

When synapsis was allowed to proceed in animals carrying the matching integrated arrays on non-homologous chromosomes, the meiotic configuration of individual nuclei was more variable, likely due to the competition between the endogenous PCs and the integrated arrays (Fig. 6i; Supplementary Information, Table S4). In 71% of pachytene-stage nuclei,

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the integrated arrays were together and the associated non-homologous chromosomes were synapsed, while the true homologous partners of the two array-bearing chromosomes remained unsynapsed. 10% of nuclei showed complete homologous synapsis with the integrated arrays apart, indicating that the arrays occasionally failed to induce non-homologous synapsis even if they had initially paired. An additional 6% of nuclei showed complete synapsis, yet the integrated arrays were closely associated. This may reflect physical association between two pairs of homologously synapsed chromosomes; alternatively the normal copies of *V* and *X* may have synapsed with each other, or aberrantly loaded SC without pairing.

Together, these data suggest that the artificial PCs created by integrated ZnF-binding arrays have potent pairing activity and that they can sometimes, but not always, mediate synapsis between non-homologous chromosomes even in the presence of homologous partners carrying intact PCs. These results are consistent with the idea that the integrated arrays and endogenous PCs compete to initiate synapsis. We emphasize, however, that the integrated arrays have important differences from endogenous PCs. Not only are the repeats probably present at both higher density and much higher copy number within the artificial PCs, but within natural PCs the ZnF-recruiting motifs are distributed among hundreds of kilobases of chromosome-specific sequences. We consider it likely that these interspersed sequences play an important role in specifying partner choice, perhaps by stabilizing and/or destabilizing pairing. This would explain why pairing between ZIM-1 or ZIM-3 binding PCs on different chromosomes is not detected in wild-type animals[8].

ZIM/HIM-8 recruiting arrays induce meiotic defects

Consistent with the aberrant interactions observed between arrays on non-homologous chromosomes, animals carrying free HIM-8 and ZIM recruiting arrays exhibited meiotic defects, including unsynapsed chromosomes, univalents, and male and inviable progeny (Supplementary Information, Fig. S6). These defects may reflect aberrant pairing between arrays and endogenous PCs dependent on the same ZnF protein or titration of the ZIM/HIM-8 proteins off the endogenous PC onto the array. However, these are unlikely to be the only explanations, since other chromosomes also showed segregation defects; for example, ZIM-binding arrays induced a weak Him phenotype, indicating missegregation of the *X* chromosome. This suggests that the arrays may titrate limiting components, such as nuclear envelope proteins, away from endogenous PCs that do not share the same ZnF dependence.

These findings help to illuminate the mechanisms underlying homolog pairing, synapsis, and segregation during meiosis. They demonstrate that PCs are likely defined by the binding of ZIM/HIM-8 proteins, via a novel, composite DNA binding domain, to a concentrated locus on each chromosome, and that consequent association with a complex of proteins at the nuclear envelope is both necessary and sufficient to facilitate specific pairing and synapsis between homologous chromosomes.

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Online Methods

SNP Mapping

The boundaries of the deficiencies *meDf2, meDf3, meDf5*, and *yDf19* were mapped using "snip-SNPs," polymorphisms that alter a restriction site between N2 Bristol and a Hawaiian isolate of *C. elegans*[12, 13]. An *unc-1 dpy-3* strain with Hawaiian-derived SNPs to the left of *dpy-3* was generated by extensive outcrossing to the Hawaiian strain CB4856. *mnDp66; Df* males were then crossed to these Hawaiian *unc-1 dpy-3* hermaphrodites. Cross progeny were allowed to self-fertilize, and Unc nonDpy F2s (lacking *mnDp66*, which carries N2 SNP alleles) were lysed and their DNA was genotyped for seven SNPs ranging from 0.05 to 2.07 Mb from the left end of the *X* chromosome. Detection of only the Hawaiian digest pattern indicated that a SNP lies within the deletion, whereas both N2 and Hawaiian alleles were expected for SNPs outside the deletion.

Progeny analysis

Hermaphrodites were picked to individual plates as L4s and moved daily to fresh plates until they no longer laid eggs. All of their self-progeny were counted to determine the proportion of unhatched (dead) eggs and adult males and hermaphrodites. The following strains were analyzed: *ieEx69* (ZIM-3 recruiting array derived from the PC region of Chromosome *I;* n=1370), *ieEx29* (ZIM-1 recruiting array derived from an amplicon in the PC region of Chromosome *II;* n=1754), *ieEx41* (HIM-8 recruiting array generated from PC region of *X* chromosome; n=3170), wild-type (n=1954), *mnDp66; meDf2* (n=2217), *mnDp66; unc-119(ed3); meDf2 ieIs5* (n=3637), *ieIs14* (n=2370), *ieIs15* (n=1663), *him-8(mn253)* (n=1315), *him-8(mn253); ieIs14* (n=1526), and *him-8(mn253); ieIs15* (n=1111).

Extrachromosomal Arrays and Integration

Extrachromosomal arrays were generated by injecting DNA mixtures including a plasmid carrying a phenotypic marker (*rol6(su1006), unc-119*(+), *or myo-2*::GFP) at 10-100 μg/ml, phage λ DNA (HindIII digested; New England Biolabs) at 50-100 μg/ml, and candidate DNA sequences on cosmids, plasmids, or PCR products, at 10-50 μg/ml. PCR primers are listed in Table S1.

A synthetic 84 bp oligonucleotide consisted of four tandem copies of the sequence AATTTGTGTTGGTCAGTGCAA. Both DNA strands were synthesized, annealed and co-injected with plasmids carrying phenotypic markers. Similar results were obtained when the same sequence was cloned into a TOPO vector (Invitrogen).

To integrate a HIM-8 recruiting array containing a 539 bp amplicon from cosmid K06A9 (*ieEx22*), array-bearing animals were crossed to the *X* chromosome PC deficiency strain *mnDp66 (X;I); unc-119(ed3) III; meDf2 X. ieEx22* contains an *unc-119* rescuing construct, so the resulting array-bearing, *meDf2* F2 animals were nonUnc and Him. The worms were washed 4x in M9 and placed on a 10-cm unseeded NGM plate. Once the liquid had absorbed into the agar, the plate was placed uncovered into a Stratalinker, and irradiated with 350 J/m^2 of 254-nm light. The animals were allowed to recover for four hours on food. Four L4s or young adults were then transferred to each of 40 plates. When their progeny reached the

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L4 larval stage, 500 of these F1s were picked to individual plates. From each F1, three F2 animals were picked to individual plates and their progeny (F3s) were screened for stable transmission of the nonUnc phenotype. A single integrant that mapped to the *X* chromosome was recovered. This array-containing *X* chromosome was outcrossed three times to *meDf2* animals to generate the *mnDp66 (X;I); unc-119(ed3) III; meDf2 ieIs5 X* strain used for analysis.

Because a duplication of the *X* PC region, *mnDp66*, was present in the original irradiated animals, we checked to make sure that the improvement in segregation was not a result of this duplication recombining back onto the *X* chromosome. The *unc-1* locus is present on *mnDp66* and absent from *meDf2*. The *dpy-3* locus is present on *meDf2* and absent on *mnDp66*. We therefore crossed *mnDp66; meDf2 ieIs5* hermaphrodites to N2 males, and mated the resulting male cross progeny, *mnDp66/+; meDf2 ieIs5*, to *unc-1 dpy-3* hermaphrodites. Unc nonDpy progeny (*unc-1 dpy-3/meDf2 ieIs5*) were recovered at the expected frequency of 50%, indicating that the *unc-1* region is still missing *from the meDf2 ieIs5 X* chromosome.

Chromosomal integration of a ZIM-2/HIM-8 recruiting array was performed similarly to the integration of the HIM-8 recruiting array above, except that the extrachromosomal array was derived from a region of sequence motifs from Chromosome *V* with 18 bp spacing and carried a dominant *rol-6(su1006) marker* and except for the presence of the array (*ieEx75*), the parental animals carried a normal karyotype. 230 F1s were individually plated after UV-irradiation. Ten lines were identified that produced 100% Rol progeny, of which two mapped to the left arm of Chromosome *V* (*ieIs12* and *ieIs13*) and two mapped to the right arm of the *X* chromosome (*ieIs14* and *ieIs15*).

Immunofluorescence and FISH

Cytological methods were performed as previously described[9]. A FISH probe specific for the extrachromosomal and integrated arrays was synthesized from λ-phage DNA as previously described[8]. Chromosome-specific probes recognizing the middle and right ends of the *X* chromosome and to the 5S rDNA on Chromosome *V* have been described previously[9, 28].

To quantify the frequency of recombinant chromosomes, hermaphrodites were picked as L4s and maintained at 15°C for three days. Adults were dissected, fixed, and hybridized with appropriate fluorescent probes to allow unambiguous identification of the relevant chromosomes. 3D images of oocyte nuclei at diakinesis were recorded and chromosomes were scored as bivalent (both homologs connected) or univalent (separate). The number of nuclei scored for each experiment were as follows: *X* chromosomes in wildtype (n=162), *mnDp66; meDf2* (n=121), *mnDp66; unc-119(ed3); meDf2 ieIs5* (n=174), *ieIs14* (n=157), *ieIs15* (n=110), *him-8(mn253)* (n=150), *him-8(mn253); ieIs14* (n=238), and *him-8(mn253); ieIs15* (n=300). Chromosome *V* in the following strains: wild-type (n=194), *ieIs12* (n=105), *ieIs13* (n=115), *zim-2(tm574)* (n=140), *zim-2(tm574); ieIs12* (n=155), and *zim-2(tm574); ieIs13* (n=127). For all experiments involving integrated arrays, an array-specific probe was included to confirm that the chromosome being analyzed (*i.e.*, *X* or *V*) contained an array.

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Feeding RNAi

To examine chromosome pairing in the absence of synapsis, expression of *syp-2*, which encodes essential component of the central element of SC, was eliminated by feeding RNAi in *mnDp66; unc-119(ed3); meDf2 ieIs5* and *mnDp66; unc-119(ed3); him-8(tm611); meDf2 ieIs5* animals, and also in the *trans*-heterozygous progeny of [*ieIs12 or ieIs13*] and [*ieIs14 or ieIs15*]. L4 larvae were placed on plates containing IPTG with lawns of bacteria containing RNAi clone sjj_C24G6.1[29]. Their progeny were dissected and stained 20-24 hours post-L4. To examine cross-progeny of animals carrying arrays on different chromosomes, matings were carried out on *syp-2* RNAi lawns and the resulting transheterozygotes were maintained on *syp-2* RNAi plates until dissection, 20-24 hours post-L4. Absence of synapsis was verified by SYP-1 immunofluorescence in all analyzed animals.

Computational Identification of Motifs

To determine if sequences related to TTGGTCAGTGCT might also be enriched on the *X* chromosome, a Perl script was written to search the genome, allowing variation at one nucleotide within the sequence at a time. The script was run iteratively to analyze any related sequences that were enriched at least 5-fold on the *X* chromosome relative to the autosomes. All such derived, enriched *X* chromosome motifs are presented in Supplementary Information, Fig. S1a. The same method was used to identify sequences related to the ZIM recruitment motifs that are enriched on the autosomes (see Supplementary Information, Fig. S2a, Fig. S3a, and Fig. S4a).

SELEX

In vitro site selection by SELEX was performed exactly as described[24]. Fragments of ZIM/HIM-8 cDNA constructs were PCR-amplified, in a two-step scheme, to yield linear products carrying HA-tags at the carboxy-terminus. A library of DNA fragments carrying a 21 bp randomized sequence flanked by a constant region was used for experiments on ZIM-2 and ZIM-3, whereas two libraries, one with a 21 bp and the other a 26 bp randomized portion, was used for experiments on HIM-8 (data presented are collate findings from both). The MEME server[30] was used to identify motifs and generate the logograms presented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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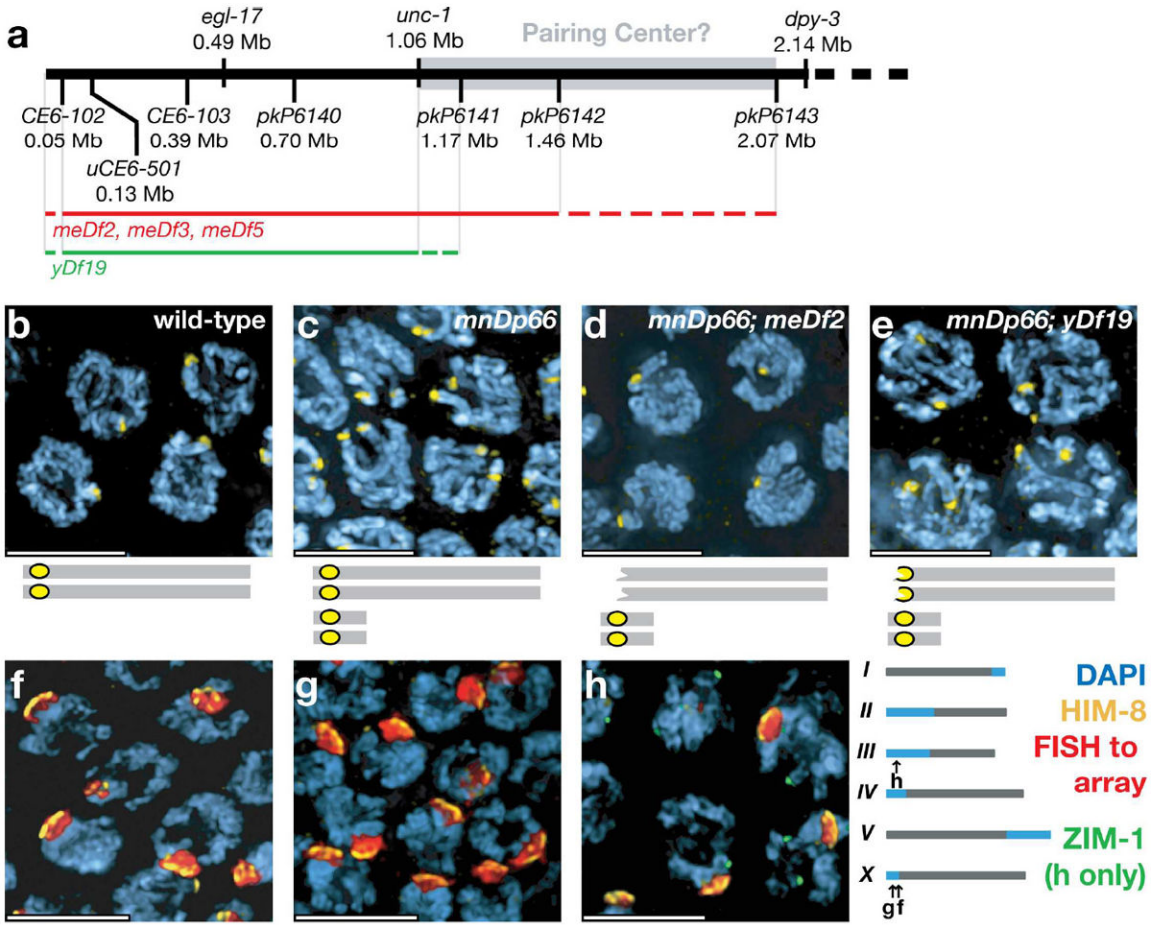

Figure 1.

The *X* chromosome PC region. (**a**) Left two megabases of the *X* chromosome. Genetic and physical markers used for mapping are indicated. Three deficiencies that remove the PC (*meDf2*, *meDf3*, and *meDf5*) and one that does not (*yDf19*) were mapped. All PC deficiencies remove *pk6142* but not *pk6143*, indicating breakpoints between 1.46 and 2.07 Mb from the left end. *yDf19* removes *unc-1* but not *pk6141*, indicating a breakpoint between 1.06 and 1.17 Mb from the left end. (**b-e**) HIM-8 immunofluorescence (yellow) in meiotic nuclei from hermaphrodites of the indicated genotypes. Diagrams of the *X* chromosomes and the *mnDp66* duplication, which is required for viability in deficiency homozygotes, are shown. (**f-h**) HIM-8 immunofluorescence (yellow) was combined with FISH (red) to test for recruitment of HIM-8 to extrachromosomal arrays. Diagram on right indicates the genomic location of the sequences tested in each panel: (**f**) 539 bp amplicon from cosmid K06A9 on *X*L. (**g**) Cluster of TTGGTCAGTGCA repeats from *X*L. (**h**) Cluster of 4 HIM-8 recruitment motifs from *III*L recruits HIM-8 (yellow) but not ZIM-1 (green). All images are maximum-intensity projections of deconvolved 3D stacks. Scale bars represent 5 μm.

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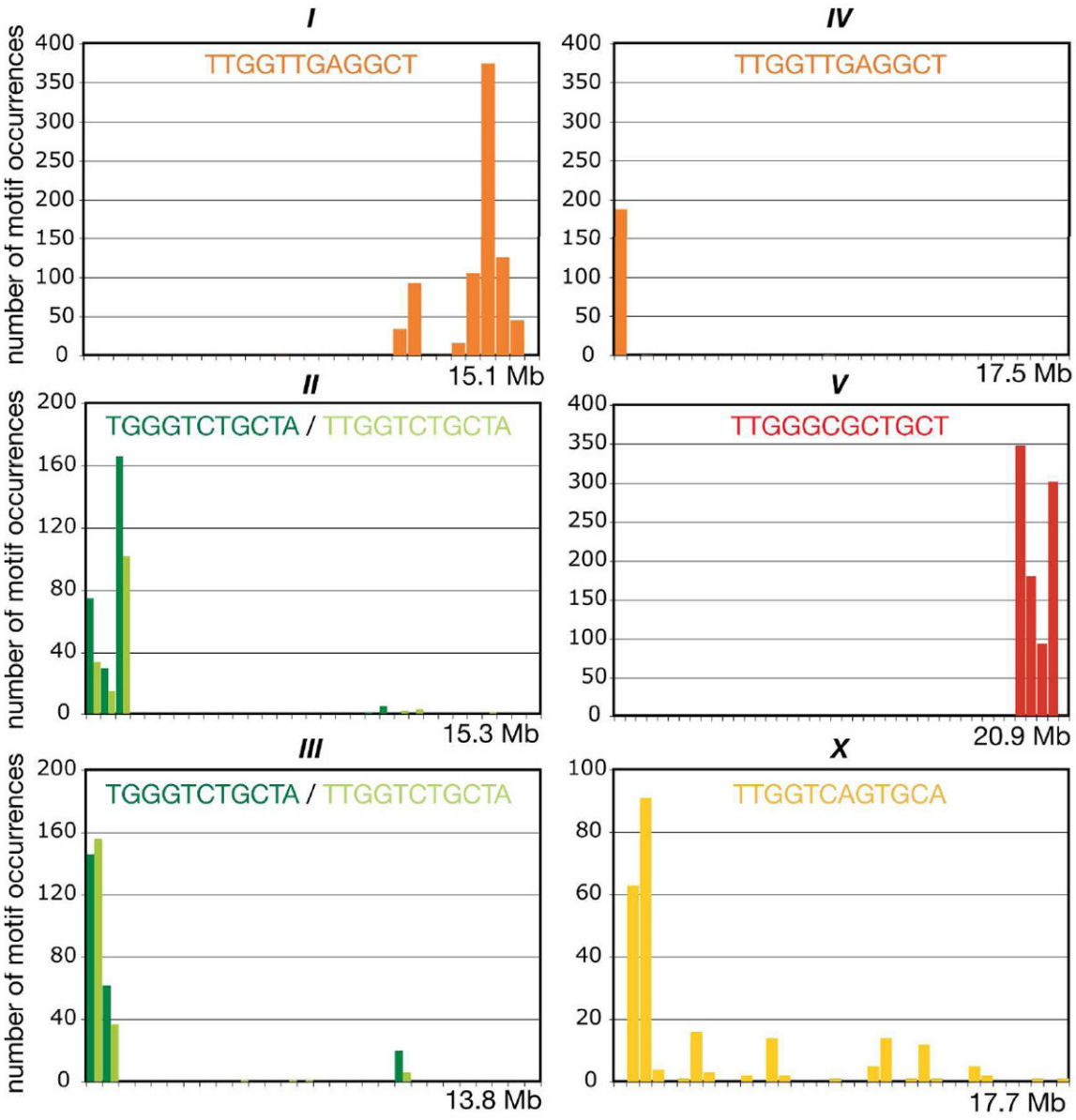

Figure 2.

ZIM/HIM-8 recruitment motifs. Distribution of the most abundant ZIM-1 (green), ZIM-2 (red), ZIM-3 (orange), and HIM-8 (yellow) recruitment motif on the six *C. elegans* chromosomes. Each bin along the X axis represents a 500 kb genomic segment. Note that Y axes show different scales for each chromosome.

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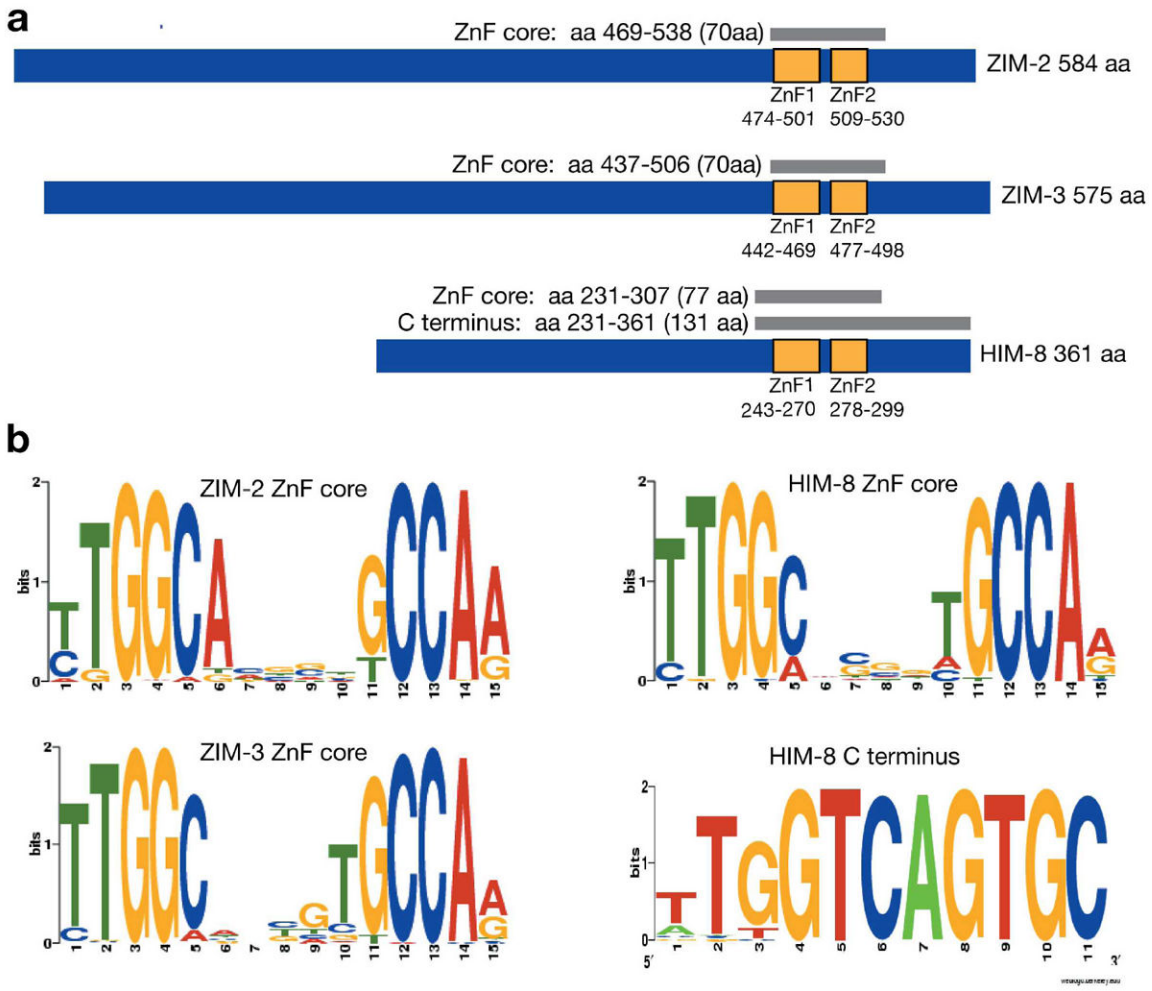

Figure 3.

Sequence-specific binding by ZIM-2, ZIM-3, and HIM-8 protein fragments. (**a**) Diagram of ZIM-2, ZIM-3, and HIM-8 showing the full-length proteins (blue), the position of the zinc fingers (ZnF, gold boxes), and the protein fragments expressed *in vitro* and subjected to SELEX analysis (gray bars). (**b**) Consensus motifs derived using MEME from the unique sequences identified following four iterations of SELEX (Supplementary Information, Table S3). Protein fragments containing the core ZnF domains of each protein show specific binding to the DNA sequence TTGGC. The randomized region in the library used for SELEX is 21-26 bp, which is significantly larger than the stretch that a 2-finger module can specify. For this reason, oligonucleotides containing two binding sites are more likely to be co-precipitated with tagged proteins than sequences containing a single site (E. Rebar, pers. comm.). A C-terminal fragment of HIM-8, including the ZnF domains, specifically binds to the same sequence identified by recruitment of HIM-8 to extrachromosomal arrays.

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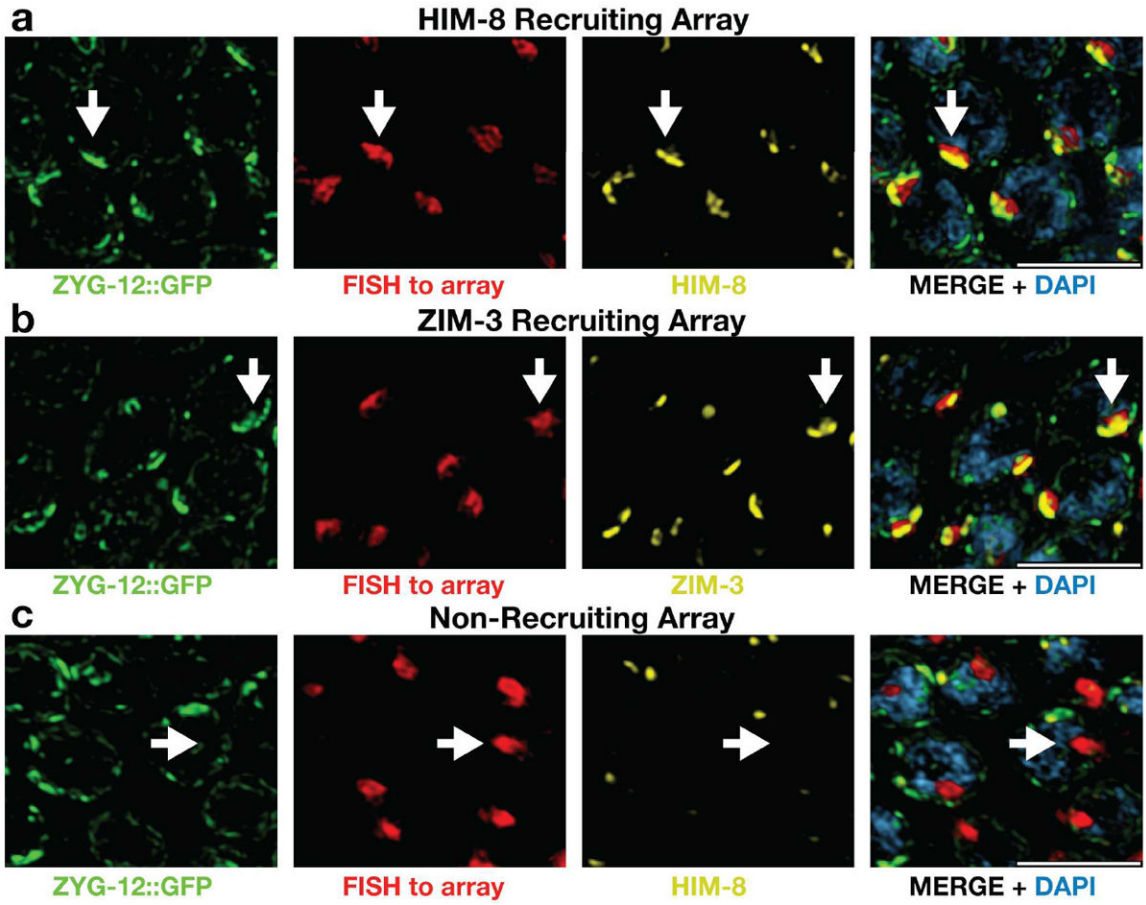

Figure 4.

ZIM/HIM-8 recruiting arrays associate with nuclear envelope components. (**a-c**) Combined FISH detection of extrachromosomal arrays (red) and immunolocalization of HIM-8 or ZIM-3 (yellow) and ZYG-12:GFP (green). HIM-8 (**a**) and ZIM-3 (**b**) recruiting arrays interact with a large patch of ZYG-12 at the nuclear envelope. The array shown in (**c**) does not recruit HIM-8 or any of the ZIM proteins, and does not colocalize with ZYG-12. Arrows indicate clear examples of co-localization (or lack thereof, in **c**). All images are maximum-intensity projections of deconvolved 3D stacks. Scale bars represent 5 μm.

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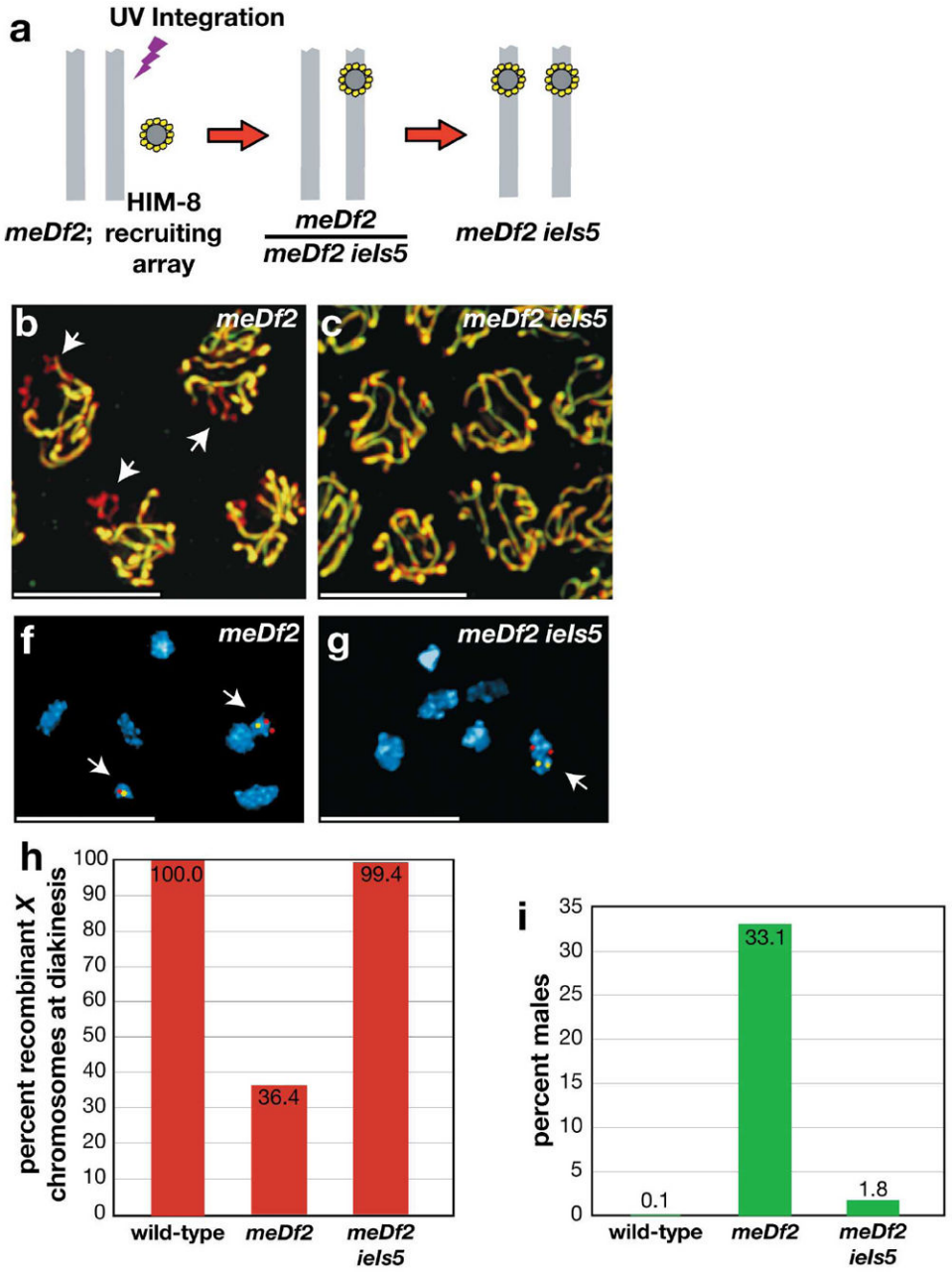

Figure 5.

HIM-8 recruitment motifs are sufficient for PC function. (**a**) Integration of a HIM-8 recruiting array (see Fig. 1f) onto *meDf2*, a PC-deficient *X* chromosome. (**b**) Hermaphrodite homozygous for *meDf2* has unsynapsed *X* chromosomes in most pachytene nuclei, visualized as axial elements marked by HTP-3 (red) lacking the central element protein SYP-1 (green). Arrows indicate examples of unsynapsed chromosomes. (**c**) Most meiotic nuclei in *meDf2 ieIs5* hermaphrodites are fully synapsed. (**d, e**) Stabilization of pairing in the absence of synapsis (*syp-2* RNAi) is examined by performing FISH to the integrated array (red). In *meDf2 ieIs5* oocytes (**d**) the arrays are paired, as indicated by only a single region of FISH staining. In *him-8; meDf2 ieIs5* oocytes (**e**) the arrays are unpaired,

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indicating that pairing between integrated arrays, like that seen between endogenous PCs[9], is *him-8* dependent. (**f, g**) Oocytes at diakinesis in *meDf2* and *meDf2 ieIs5* hermaphrodites. FISH probes to the center (yellow) and right end (red) identify the *X* chromosomes. Arrows indicate non-recombinant (univalent) and recombinant (bivalent) *X* chromosomes in *meDf2* and *meDf2 ieIs5* hermaphrodites, respectively. (**h**) Quantification of recombinant *X* chromosomes. (**i**) Quantification of males produced by self-fertilizing hermaphrodites of the indicated genotypes. Scale bars represent 5 μm.

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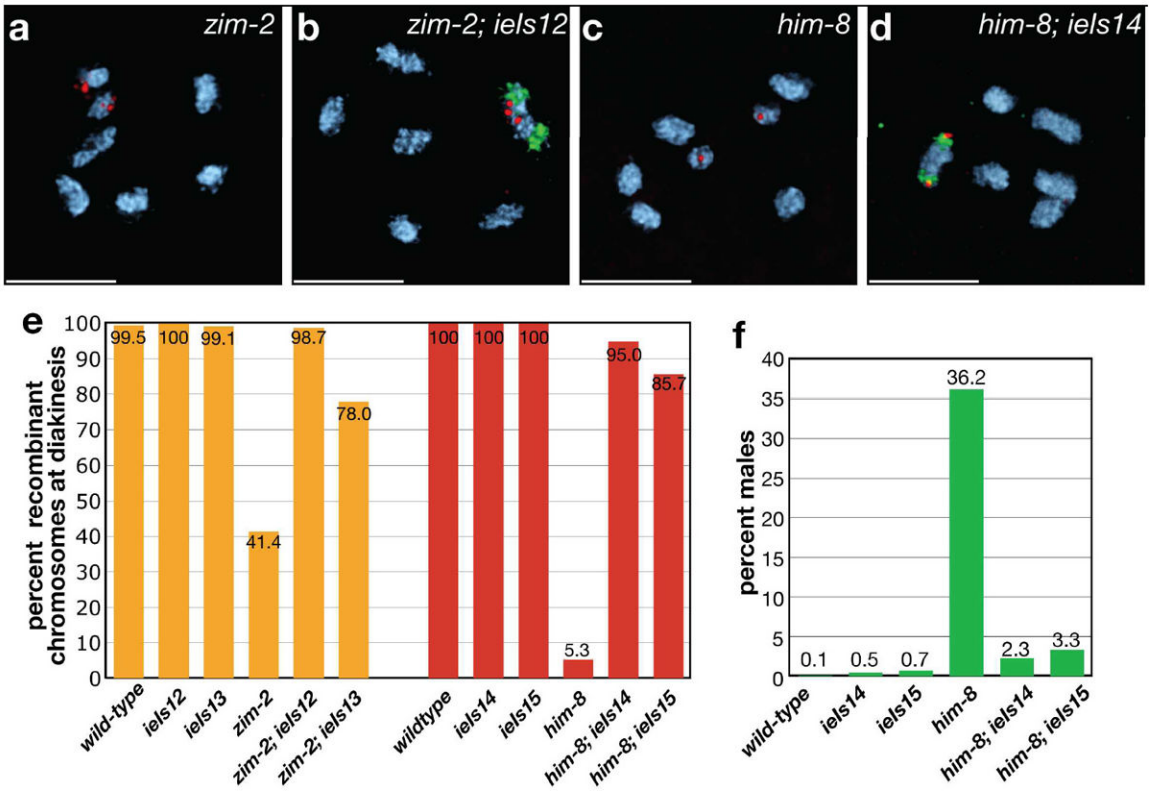

Figure 6.

ZIM/HIM-8 proteins can interchangeably support PC function. (**a-d**) Oocytes at diakinesis from [*zim-2*], [*zim-2; ieIs12*], *[him-8]*, and *[him-8; ieIs14]* hermaphrodites. FISH probes to the 5S rDNA (red in **a, b**) and an *X*-chromosome repeat (red in **c, d**) were used to identify chromosomes *V* and *X*, respectively. In animals carrying chromosomal insertions of ZIM-2/HIM-8 binding sites *ieIs12* (**b**) and *ieIs14* (**d**), the chromosome-specific probes localize to a single bivalent, which is also marked by a FISH probe to λ DNA (green), indicating that the insertion of binding sites restored crossover recombination on chromosome *V* in *zim-2* animals and the *X* chromosome in *him-8* animals. (**e**) Quantification of bivalent *X* (red) and *V* (orange) chromosomes. (**f**) Quantification of males produced by self-fertilizing hermaphrodites of the indicated genotypes. (**g,h**) Arrays of binding sites (red) inserted into two different chromosomes *(ieIs12/+; ieIs14/+* and *ieIs13/+; ieIs15/+)* were assayed for their ability to promote stable pairing between non-homologous chromosomes in the absence of synapsis (*syp-2* RNAi). (**i**) Synapsis was analyzed in animals heterozygous for two different insertions (*ieIs13/+; ieIs15/+*) by immunostaining of SC components. Nuclei containing unsynapsed chromosomes, visualized as segments positive for the axial element protein HTP-3, (red) but lacking transverse filament proteins including SYP-1 (green), usually contain integrated arrays (blue) that are paired and synapsed with each other, indicating non-homologous synapsis between chromosomes *V* and *X* (arrows). In contrast, nuclei with fully synapsed chromosomes often contained unpaired arrays (blue), indicating that all chromosomes are likely synapsed with their appropriate homologs (arrowheads). Scale bars represent 5 μm.