

Chan et al. <http://www.jcb.org/cgi/doi/10.1083/jcb.200408061>

## Supplemental materials and methods

### Antibody production

The following peptides were used for the production of antibodies in rabbits and rats: DPY-26 carboxyl terminus, CGGQPTSDLGAIVEEEEMEE; DPY-28 carboxyl terminus, CKKSAVADDSDSDEFMLDD; HCP-3 amino terminus, ADDTPIIEEIAEQNESVTRIMQRLKHDMQR; and HCP-6 carboxyl terminus, CGKEDLKNRKSQAYHLHNIFEDEEEES.

### Templates for dsRNA production

Templates for in vitro transcription were PCR amplified from genomic DNA using the following primer sets. T7 and T3 RNA polymerase promoter sequences are underlined. *hcp-6*, 5'-GCGTAATACGACTCACTATAGGGTCGTGAAAACTCGTCAAGCG CGCAATTAACCCTCACTAAAGGGTGCAAT-TCTGGATGGTCTAGC-3'; *mix-1*, TAATACGACTCACTATAGGGTGATACTTACGCGCTTTCGGAC TAATACGACTCACTATAGGGACTTTGAATATCTTC-GAGTTAGCCG-3'; *hcp-3*, 5'-AAITTAACCCTCACTAAAGGGTTTCGACCAAAAATGCTCC TAATACGACTCACTATAGGGATGTCGTCGCGTATTCCC-3'; F54C8.2, 5'-TAATACGACTCACTATAGGTTTAATCAGAGATTGGAAGGCA TAATACGACTCACTATAGGTTCAAAGTGCGAAAAATTGATT-3'.

## References

- Alpi, A., P. Pasierbek, A. Gartner, and J. Loidl. 2003. Genetic and cytological characterization of the recombination protein RAD-51 in *Caenorhabditis elegans*. *Chromosoma*. 112:6–16.
- Colaiácovo, M.P., A.J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo, A. La Volpe, and A.M. Villeneuve. 2003. Synaptonemal complex assembly in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev. Cell*. 5:463–474.

Table S1. Embryonic lethality in *hcp-6(mr17ts)*

Temperature	Lethality	<i>n</i>
15°C	9%	1035
25°C	98%	430

Fewer than 1% of wild-type embryos are dead at either temperature.

Table S2. Oocyte cellularization occurs relatively normally in *hcp-6(mr17ts)*

Genotype	Oocytes per gonad arm	Gonad arms scored	SEM
wild type	10.0	53	0.5
<i>hcp-6(mr17ts)</i>	8.6	68	0.3

The number of oocytes per gonad arm was counted in age-matched wild-type and *hcp-6* mutant animals.

Table S3. HCP-6 is required to remove or prevent the introduction of recombination-independent linkages between homologues.

Genotype	DAPI-staining figures per oocyte (%)							Oocytes scored
	6	7	8	9	10	11	12	
<i>spo-11(me44)</i> , 15°C	0	0	0	9	18	28	45	98
<i>spo-11(me44)</i> , 25°C	0	0	1	9	19	26	45	98
<i>hcp-6(mr17ts);spo-11(me44)</i> , 15°C	0	0	1	2	21	20	55	206
<i>hcp-6(mr17ts);spo-11(me44)</i> , 25°C	11	14	8	15	21	16	15	158
<i>spo-11(ok79)</i> , 20°C	0	0	0	0	27	32	40	77

The SPO-11 endonuclease creates double-strand DNA breaks that are required for the initiation of recombination. In *spo-11* mutants, recombination fails and homologues are achiasmate univalents in diakinesis. In *hcp-6(mr17ts);spo-11(me44)* double mutants grown at 25°C, the precocious separation of homologues is reduced relative to double mutants grown at 15°C and *spo-11(me44)* single mutants grown at either temperature, suggesting that HCP-6 is required to remove or prevent the introduction of linkages between homologous chromosomes. Importantly, the disruption of recombination by *spo-11(me44)* was not temperature sensitive because this mutation resulted in similar levels of premature homologue separation in animals grown at 15°C and at 25°C. Moreover, the level of precocious homologue separation caused by *spo-11(me44)* was similar to that of the known null allele *spo-11(ok79)*. Together with our finding that RAD-51 foci do not form on chromosomes of *spo-11(me44)* mutants, the similar phenotypes of *spo-11(me44)* and *spo-11(ok79)* mutants suggest that *me44* prevents DSBs and acts either as a null allele or a severe loss-of-function allele. Despite the apparent absence of recombination in *spo-11(me44)* and *spo-11(ok79)* mutants, the expected complement of 12 univalents was seen in less than half of oocytes. This discrepancy reflects our conservative scoring; when two univalents overlapped in space, they were scored as one DAPI-staining body.