Meiotic gene expression initiates during larval development in the sea urchin

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Abstract

Background—Meiosis is a unique mechanism in gamete production and a fundamental process shared by all sexually reproducing eukaryotes. Meiosis requires several specialized and highly conserved genes whose expression can also identify the germ cells undergoing gametogenic differentiation. Sea urchins are echinoderms which form a phylogenetic sister group of chordates. Sea urchin embryos undergo a feeding, planktonic larval phase in which they construct an adult rudiment prior to metamorphosis. Although a series of conserved meiosis genes (e.g. *dmc1, msh5, rad21, rad51,* and *sycp1*) are expressed in sea urchin oocytes, we sought to determine when in development meiosis would first be initiated.

Result—We surveyed the expression of several meiotic genes and their corresponding proteins in the sea urchin *Strongylocentrotus purpuratus*. Surprisingly, meiotic genes are highly expressed not only in ovaries but beginning in larvae. Both RNA and protein localizations strongly suggest that meiotic gene expression initiates in tissues that will eventually give rise to the adult rudiment of the late larva.

Conclusions—These results demonstrate that broad expression of the molecules associated with meiotic differentiation initiates prior to metamorphosis and may have additional functions in these cells, or mechanisms repressing their function until later in development, when gametogenesis begins.

Keywords

Meiosis; germ line; sea urchin; adult rudiment

Introduction

Somatic cells divide and multiply in a process called mitosis, whereas meiosis is unique to germ cells. Meiosis produces haploid gametes from diploid cells by combining two successive rounds of nuclear division, a reductive division (meiosis I) and an equal division (meiosis II), with a single round of DNA replication (Petronczki et al., 2003). Meiosis is critical for producing genetic variability in the gametes and the mechanisms and molecules involved in meiosis are highly conserved among all sexually reproducing organisms.

Meiosis I is initiated by recombining homologous chromosomes through the formation of chiasmata, which physically connect homologous chromosomes at the leptotene stage of prophase I (Petronczki et al., 2003; Neale and Keeney, 2006; Kleckner, 2006). This meiotic homologous recombination is initiated by the formation of double-strand breaks (DSB) created by the topoisomerase II–like protein SPO11 (Keeney, 2001) and by subsequent

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invasion and pairing of the homologous strand (Kenny, 2001) mediated by RAD51 and DMC1 (Schwacha and Kleckner, 1997; Hunter and Kleckner, 2001; Keeney, 2001). In mammalian meiosis, early recombination intermediates containing RAD51 and DMC1 are gradually replaced first by middle intermediates containing MSH4 (a homologue of *Escherichia coli* MutS) and eventually by late intermediates containing MLH1 (a MutL homologue) (Plug et al., 1998; Santucci- Darmanin et al., 2000). RAD51 and DMC1 are highly conserved in eukaryotes (Shinohara et al., 1992; Bishop et al., 1992; Shinohara et al., 1993; Habu et al., 1996) and have been shown to broadly catalyze homologous pairing and strand exchange selectively in sexually producing organisms (Sung, 1994; Baumann et al., 1996; Gupta et al., 1997; Li et al., 1997; Hong et al., 2001; Sehon et al., 2004; Bugreev et al., 2005). The RAD51 protein is expressed in both meiotic and mitotic cells (Shinohara et al., 1992 and 1993), but the DMC1 protein is only present in meiotic cells of all organisms examined (Bishop et al., 1992; Habu et al., 1996). Knockout of the dmc1 gene in the mouse causes chromosomal asynapsis and sterility (Pittman et al., 1998; Yoshida et al., 1998), as in Dmc1-deficient yeast (Bishop et al., 1992). These data suggest that the DMC1 protein functions as a specific factor for meiotic homologous recombination (HR). Another critical factor for HR is HIM-18, an ortholog of MUS312/Slx4m that is identified in *Caenorhabditis elegans*. HIM-18 is required for HR–mediated repair at stalled replication forks and its loss results in a reduction in crossover recombination frequencies (Saito et al., 2009).

At the zygotene stage of prophase I, homologs that have completed HR come into close proximity and the synaptonemal complex (SC), a zipper-like proteinaceous structure, assembles between the aligned homologs (Zickler and Kleckner, 1999; Page and Hawley, 2004). At the pachytene stage of prophase I, the homologs become fully synapsed by the SCs and repair of a subset of DSBs results in crossover recombination. SCs are largely composed of SYCP1, SYCP2 and SYCP3. In mammals, SYCP1 constitutes the central element, and SYCP2 and SYCP3 form the lateral elements of the SC (Meuwissen et al., 1992; Dobson et al., 1994; Offenberg et al., 1998). In SYCP1-deficient mice, neither synapsis nor MLH1 foci are formed (de Vries et al., 2005), suggesting its essential role in meiotic progression. At the diplotene stage of prophase I, the SCs are disassembled and the homologs undergo desynapsis in preparation for subsequent chromosome segregation during metaphase. Other factors involved both in meiotic and mitotic chromosome dynamics include SMC subunits RAD21 and SA/STAG, which are found in cohesin complexes, are important for sister chromatid cohesion and are essential for maintaining the structural integrity of chromosomes (Hirano, 2006; Nasmyth and Haering, 2009).

Most studies of meiotic mechanisms in the field result from experimentation on a few genetically tractable organisms; yeast, flies, worms, mice, providing a very deep but narrow view of how the meiotic mechanisms function in a variety of developmental and reproductive contexts. Echinoderms are a sister group to chordates and most of the conserved meiotic genes present in vertebrates are present in the sea urchin genome (Song et al., 2006). However, the developmental timing and the location of germ cell meiotic initiation and/or the expression of these meiotic genes is unknown in this phylum. A typical echinoderm undergoes a planktonic larval phase for several weeks or months and forms an adult precursor structure called the 'adult rudiment' in the left side of its larval body before metamorphosis (Fig. 1, Day 20–30 pluteus larva).

In the sea urchin, primordial germ cells are considered to reside within the left adult rudiment at metamorphosis as judged by their characteristic cytology. Yet a clear appearance of germ cells in the germ rings where the gametes form occurs several months after metamorphosis (McBride, 1903; Houk and Hinegardner, 1980). The complex mixture of multiple cell lineages in the adult rudiment and a long-term larval stage also makes it difficult for researchers to definitively identify germ cell formation (Ransick et al., 1996;

Yajima and Wessel, 2011a). Here, we identified several conserved meiotic genes in the sea urchin *Strongylocentrotus purpuratus* and surveyed their expression patterns both at the mRNA and protein levels. Surprisingly, meiotic genes are expressed in tissues of the adult rudiment in the larva, prior to the formation of a definitive gonad. These data suggest that meiotic genes may have broad utilization in somatic cells of the adult rudiment and may be linked to the plasticity of a germ cell commitment step initiated before metamorphosis.

Results and Discussion

Temporal expression of Meiotic genes in S. purpuratus

Six meiotic genes that are highly conserved among metazoans were cloned from *S. purpuratus* ovary cDNAs by PCR for lengths of 0.5-1kbp. These genes are *Sp-sycp1L* (SPU_021319)*, Sp-dmc1* (SPU_027921)*, Sp-msh4* (Glean3_25763)*, Sp-msh5*(SPU_004494)*, Sp-rad21* (SPU_002553) and *Sp-rad51* (SPU_009590), and which are each essential and selective for germ cells in early meiotic initiation. The PCR products for each gene were sequenced and BLASTed against the database at SpBase.org, which confirmed their meiotic gene identity (Table 1 and 2). By using the same primer sets and the same PCR conditions, expression of these meiotic genes was compared among cDNAs of ovary, Day10 larvae, and Day40 larvae. All six genes were highly expressed in ovaries (Fig. 2, O) but were undetectable in embryos and early larvae (Fig. 2, D10), except for *Sp-dmc1*. Remarkably, when the adult rudiment was formed at Day 40, all genes except for *Sp-msh4* demonstrated significant expression (Fig. 2, D40). In this late stage, several non-specific bands were often amplified and thus a nested PCR was performed further to isolate target genes, which resulted in a single band for each gene (data not shown). Sequencing results further demonstrated that each nested-PCR product indeed contained the targeted genes *Sp-sycp1, Sp-dmc1, Sp-msh5, Sp-rad21* or *Sp-rad51,* suggesting that these meiotic genes reinitiate expression as early as Day 40 larvae. To be noted, we occasionally found differential sizes for some genes (e.g. *Sp-dmc1* and *Sp-msh5*) among the clones from different developmental stages. This result may reflect differential spicing of various transcripts, or unique functions of those molecules outside of meiotic cellular competence, so that it is important to identify the full sequence and the function of each gene in the future.

Lineage expression of Meiotic genes

To study the spatial expression of each meiotic gene in late larvae, DIG-RNA probes were made from each PCR product and *in situ* hybridization was performed for larvae of Days 10–50. Early larvae (data not shown) and negative controls showed no specific signal accumulation, although a high background fluorescence is apparent in the stomach (auto-fluorescence by ingested algae), whereas the late larvae consistently demonstrated a specific signal within or at the periphery of the adult rudiment (Fig. 3, arrows), suggesting that several meiotic genes are expressed in the adult rudiment.

To further test if these transcripts are actually translated, larvae were imaged using immunofluorescence with antibodies against each meiotic protein. Immunofluorescence signals against DMC1 and Rad51 antibodies, interestingly, demonstrated a specific signal as early as Day10 in the coelomic pouch, which is the origin of the adult rudiment, results that are consistent with those of RT-PCR for *sp-dmc1* (Fig. 2). A signal for DMC- was also detected at the junction between the coelomic pouch and the mouth (Fig. 4, DMC1, arrowheads), which may indicate an additional function of DMC1 outside of the adult rudiment. On the other hand, antibodies against MSH5, SYCP1 and HIM-18 exhibited specific signals only in late larvae just prior to adult rudiment formation (Fig. 4). The antibody against MSH-4 was used as a negative control here because its mRNA was not

detected by RT-PCR in early nor late larval stages (Fig. 2), and indeed its protein expression was not detected in the adult rudiment (Fig. 4, Nega Cont., arrows). High signal levels consistently found in the stomach of all larvae is due to an auto-fluorescence of algae in the stomach; however, we cannot exclude the possibility that these antibodies are detecting proteins also expressed in that tissue as well.

Meiotic gene expression starts early in the adult rudiment of the sea urchin

The adult rudiment of the sea urchin has long been enigmatic. It forms most of the adult structures such as spines, tests, tube feet, a neural and vascular system, and also the primordial germ cells (PGCs) formed at the 5th division that was originally located in the coelomic pouches, a precursor of the adult rudiment (Tanaka and Dan, 1990; Yajima and Wessel, 2011a and 2012). Further, the adult rudiment cells are contributed not only by mesodermal lineages that originally consisted of coelomic pouches including PGCs but also by the ectodermal lineage in the late larval stage when a layer of ectodermal tissue invaginates to meet the expanded left coelomic pouch at the onset of adult rudiment formation (Hyman, 1955). Adult rudiments thus apparently contain several somatic cell lineages that contribute to the adult structures. The complex mixture of multiple cell lineages in the adult rudiment makes it difficult for researchers to definitively identify germ cell formation.

In this report, we used meiotic gene products as molecular markers to identify germ cell meiotic development and found that 5 genes involved in early meiosis1 event were consistently expressed in the adult rudiment at late larval stage. Although each of the meiotic gene products may have additional roles outside of germ cells, overlapped expressions of several molecules strongly suggest that the meiotic program may start as early as the late larval stage in the adult rudiment. Further, broad tissue expression of meiotic genes is readily detectable in the adult rudiment and precursor tissues, but not generally throughout the other cells of the embryo or larvae. This gene expression is also not just a result of rapid cell divisions, since many of the embryonic cells and larval tissues do not express this same gene profile. Surprisingly, these mRNA and protein distributions were broader than documented for PGC lineages in the adult rudiment and likely include somatic cell lineages. One implication of this result is that the somatic expression of meiotic genes in the adult rudiment have additional functions independent of meiosis. However, if these genes are indeed functioning within somatic cell lineages, the nature of their somatic cell identity during rudiment formation should be investigated further with implications of germ line plasticity (see e.g. Ransick et al., 1996; Yajima and Wessel, 2011). Adult rudiment cells are highly proliferative and multipotent, and they contribute to various adult tissues, thus their cell division mechanisms may be distinct from those of other somatic cells (Hyman, 1955). Although some of the meiotic genes such as DMC1 and SYCP1 have been reported as meiosis-specific factors in other organisms, these genes may be more widely utilized for other chromosomal events of the adult rudiment cells of echinoderms. An alternative interpretation is that meiotic genes in the adult rudiment were spurious, nonfunctional transcriptional events, or are repressed from their normal activity and thus localized in the cytoplasm. In mice, for example, some of the germ-line-related molecules are widely expressed and then become more restricted only into the future PGC populations during the course of germ cell development (Saito et al., 2002 and 2003). A broader expression of meiotic molecules such as those we observed here may be similar to this case; thus, germ cell formation may be initiated in a part of the adult rudiment of late larvae. Indeed, recent reports in mice suggest that some of the meiotic genes appear to be expressed prior to the initiation of the meiotic events (Baltus et al., 2006; Kimble and Page, 2007). SYCP3, for example, initiates cytoplasmic expression in the pre-meiotic germ cells before it localizes and functions on the chromosomes during the meiosis (Lin et al., 2008). The regulatory

mechanisms of meiotic gene expressions in higher eukaryotes are still largely unknown. Further, this is the first report of meiotic gene expression in the phylum of echinoderms. Further investigation, especially in the functional contributions of these meiotic molecules in the adult rudiment, will be essential.

Experimental Procedures

Animals and larval culture

Strongylocentrotus purpuratus were obtained from Trevor Fay (trevor@montereyabalone.com, CA) and housed in aquaria cooled to 16°C in artificial seawater (ASW; Coral Life Scientific Grade Marine Salt; Energy Savers Unlimited, Inc, Carson, CA). Females were shed by KCl (0.5 M) injection and eggs were collected in Millipore filtered seawater (MFSW; Millipore USA); ovaries were removed and minced in MFSW. To obtain embryos, fertilized eggs were cultured at 16°C in MFSW and collected at necessary developmental stages. Larvae were reared after hatching (24 h after fertilization according to the method described previously (Yajima and Kiyomoto, 2006) with slight modifications. Briefly, larvae were cultured in 4L beaker with gentle stirring at 30 rpm with a rotator (TAITEC, Tokyo, Japan), fed with two types of diatoms, *Chaetoceros gracilis* and *Dunaliella tertiolecta* (UTEX, utalgae@uts.cc.utexas.edu, Texas, USA), and transferred to new MFSW twice weekly. Larvae reached the 4-armed stage after 4–21 days, the 6-armed stage after 3–5 weeks, and the 8-armed stage after 5–8 weeks at 16°C.

RT-PCR

RT-PCR was performed as described previously (Juliano et al., 2006). Gonadal tissues, 10 day and 40 day larvae of *S. purpuratus* were collected and subjected to total RNA extraction with RNeasy Mini kit (Qiagen). The RNA was made into cDNA with TaqMan RT-PCR kit (Roche), and 1 µl of each cDNA was used for PCR reactions. PCR primers for each gene are listed in Table 1. Obtained PCR products were subcloned into pGEMT-EZ vectors (Promega, Madison), inserts were sequenced either by the SP6/T7 promoter primer, and sequencing results were blasted against SpBase.org to identify gene names (Table 2).

In situ RNA hybridization

In situ hybridization was performed as previously described (Arenas-Mena et al., 2000). Ovaries and larvae were fixed in paraformaldehyde, washed with MOPS butter, transferred to 70% EtOH and stored at −20°C until use. Antisense DIG-labeled probes for *Sp-dmc1, Sp-msh5, Sp-Rad51, Sp-Rad21*, and *Sp-sycp1*, were transcribed from plasmid templates acquired through RT-PCR reactions above and in vitro transcribed with DIG-RNA labeling kit (SP6/T7) (Roche, Indianapolis). A non-specific DIG-labeled RNA probe complimentary to pSPT18 that encodes neomysin gene was used as a negative control. Hybridized probes were reacted with anti-Digoxigenin-POD (Roche #11207733910), and visualized with TSA system (Perkin Elmer). Hoechst was used as a counter-staining at a final concentration of 0.1 µg/ml, and samples were imaged by confocal microscopy (Zeiss, 510).

Immunolabeling

Immunolabeling was performed as previously described (Yajima and Wessel, 2011b; Yajima and Kiyomoto, 2006) and photomicrographs were taken with the use of confocal microscopy (Zeiss, 510). Briefly, larvae were fixed with 90% Methanol for 1hr at −20C, rinsed with PBS, exposed for the primary and secondary antibody reactions for 5hrs each. Primary antibodies used in this study were all provided by the courtesy of Drs. Monica Colaiacovo and Satoshi Namekawa. The original sources and experimental conditions of these antibodies are as follows: anti-DMC1 at 1:100 (Thermo Scientific #PA5-21472), anti-

MSH-5 at 1:100 (Novus Biologicals, #29480002), anti-RAD-51 at 1:200 (Novus Biologicals, #38750002), anti-mouse-SYCP1 at 1:200 (Abcam #ab15090), anti-*c.elegans*-SYP-1 at 1:750 (Smolikov et al., 2007), anti-HIM-18 at 1:50 (Saito et al., 2009), anti-MSH-4 at 1:200 (Abcam #ab58666). Cy3 goat anti-rabbit immunoglobulin G (IgG) antibody (Invitrogen) was then used as a secondary antibody at 1:300. Hoechst was used as a counter-staining at a final concentration of 0.1 μg/ml. Peptide alignments between *S. purpuratus* protein and the other organism used for the antibody production is listed in Table 3.

Acknowledgments

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Highlights

1. A series of meiotic gene products are expressed prior to metamorphosis in the sea urchin.

2. Meiotic gene products, both mRNA and protein, are broadly expressed in the entire adult rudiment of the sea urchin.

3. Germ cell development may be more plastic, and/or the functions of these gene products more broadly required than previously thought.
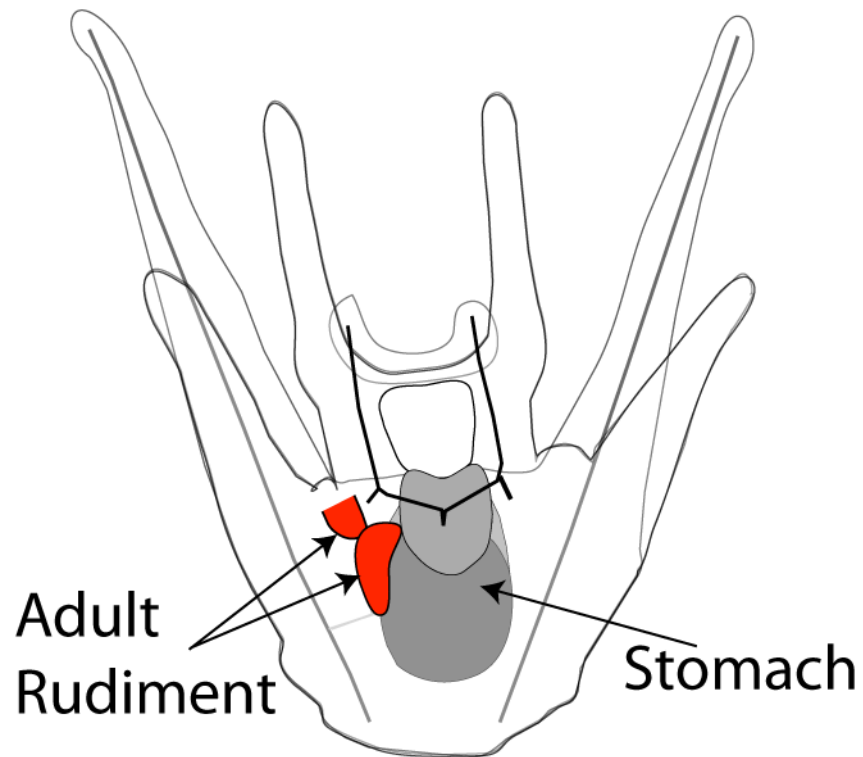
Figure 1.
A diagram of the sea urchin late larva around Day 20–30. The adult rudiment initiating in the left side of the larval body is marked in red and this undifferentiated cell populations will form most of the adult structures including tube feet, spines, tests, vascular system, nervous system, and others before metamorphosis at Day 40–50.
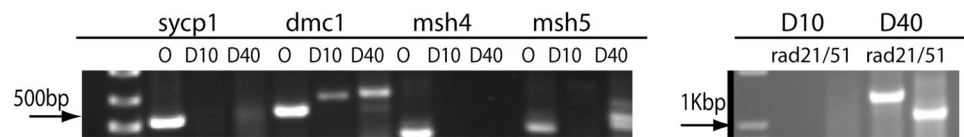
Figure 2.
Meiotic gene expression initiates in the adult rudiment of late larvae in the sea urchin. RT-PCR results indicate that a series of meiotic genes are expressed in the oocytes (O) and the late larvae (Day40, D40), but not in the early larvae (Day10, D10) except for *Sp-dmc1*.
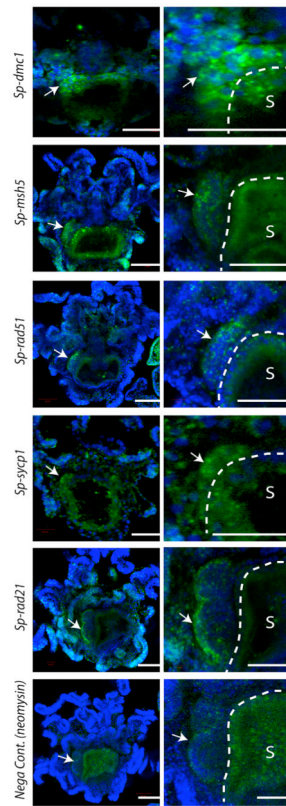
Figure 3.
Confocal images of fluorescent *in situ* RNA hybridization in late larvae (left panels), and a magnified view of the adult rudiment (right panels). A specific signal is localized within or at the periphery of the adult rudiment with meiotic probes (arrows), whereas the negative control indicates only a background signal in the stomach. Stomach (S) is outlined by a dashed-line. Meiotic genes (Green) and DNA (Blue). Scale bars=50 μm.
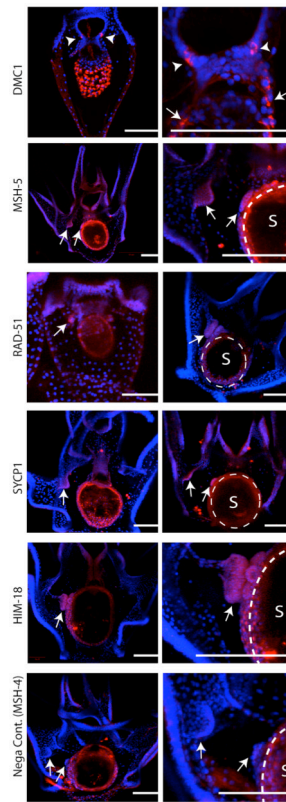
Figure 4.
Confocal images of immunofluorescence with meiotic antibodies. Each antibody, except for the negative control (MSH4, not detected by PCR, in situ hybridization, nor immunolocalization), detected a specific signal in the coelomic pouches or the adult rudiment of the late larva (arrows). Arrowheads in DMC1 indicate a junction between the coelomic pouch and the mouth. Right panels of DMC1, MSH5, Him-18 and MSH4 indicate a magnified view of each of left panels. The left panel of SYCP1 indicates *C. elegans* SYCP1 antibody and the right panel indicates Mouse SYCP1 antibody. Stomach (S) is outlined by a dashed-line. Meiotic proteins (Red) and DNA (Blue). Scale bars=50 μm.

Table 1

A list of primer sequences used for cloning of meiotic genes.

Gene product	Forward Primer	Reverse Primer
Sp-dmc1	GAATGCCATGGTTCTTTCCGTTCG	CACAAATTGTATGGGCCTGAATC
Sp-dmc1-nest	CTTTCCGTTCGTATCTACTGGTG	ATCGGGCCTGAATCTTGATTCTG
Sp-msh5	CAGGCAGATCCGTGCCACTGGAGG	GCTCGACATATCTCAGCAATATCG
Sp-msh5-nest	GCCACTGGAGGACTTCTGAAGTTC	CTCAGCAATATCGATGGCATTG
Sp-sycp1	CAAAGGGTCAGTCCTTCCAGACGC	GAGTTGAGTCTGGCTCAGTGCAAC
Sp-sycp1-nest	GTCCTTCCAGACGCAGCCCCTTC	AGACTCAACTCTTGAAACTTC
Sp-rad21	ATGGCAATGCAAATGCACAATGCTGAG	ATGGTGATGTCTCCATACGTGACGTTC
Sp-rad51	ATGGCAATGCAAATGCACAATGCTGAG	AGTCCTTGGCATCACCAACACCATC
Sp-msh4	GAAGCAGATAGCGCTGCTTCAGAT	GTTGTTCTTCCTCTTGACAGGATG

Table 2

Sequencing Results of each meiotic gene.

```
TOP  : DMC1                                                              TOP  : MSH5
Middle: Dmc1-Ovary                                                        Middle: Msh5-Ovary
Bottom: Dmc1-D40                                                          Bottom: Msh5-D40
CAAATTGTATCGGGCCTGAATCTTGATTCTGAACAGGGAAGAACTGAAGAGGACTACTAT 62          CATATCTCAGCAATATCGATGGCATTGTAGGCTGTCTTGTAGAGGGACTGCCAGTCTGTC 66
CAAATTGTATCGGGCCTGAATCTTGATTCTGAACAGGGAAGAACTGAAGAGGACTACTAT 120         ------------------------------GCTGTCTTGTAGAGGGACTGCCAGTCTGTC 30
------------------------GATTCTGAAGA--------CTGAAGAGGACTACTAT 28          CATATCTCAGCAATATCGATGGCATTGTAGGCCGTCTTGTAGAGGGACTGCCAGTCCGTC 120
                        ********* *           **************                      ** ********************** ***

CTACATCATACACATAGGAACCAAGCAGGTAGCCATCATGATCATACTCATCTCGTAATC 122        ATGGAGGCTTGTGCTTGCTGCATCTGCTTGAAGATTCTCGGAACATTCTTAACATTCTTG 126
CTACATCATACACATAGGAACCAAGCAGGTAGCCATCATGATCATACTCATCTCGTAATC 180        ATGGAGGCTTGTGCTTGCTGCATCTGCTTGAAGATTCTCGGAACATTTTTAACATTCTTG 90
CTACATCATACACATAGGAACCAAGCAGGTAGCCATCATGATCATACTCATCTCGTAATC 88         ATGGAGGCTTGTGCTTGCTGCATCTGTTTGAAGATTCTCGGAACATTTTTAACATTCTTG 180
************************************************************                ********************** ***************** ***********

CCCAGACTGTAAAGTTCTTTGGAGCACTATCTATTATTCCTGCAGGATCTAAGGCTTTGG 182        AGGCAATTCTGAAGTGACCCGGTGACCTCAGCGTTCTTAGGATTTAAGAAGAAAGAAACA 186
CCCAGACTGTAAAGTTCTTTGGAGCACTATCTATTATTCCTGCAGGATCTAAGGCTTTGG 240        AGGCAATTCTGAAGTGACCCAGTGACCTCAGCGTTCTTTGGATTCAAGAAGAAAGAAACG 150
CCCAGACTGTAAAGTTCTTTGGAGCACTATCTATTATTCCTGCAGGATCTAAGGCTTTGG 148        AGGCAATTCTGAAGTGACCCAGTGACCTCAGCATTTTTTGGATTCAAGAAGAAAGAAACA 240
************************************************************                ********************.*********.** **.***** *************

GAATGTGCTCCAAGGAGAATGCTGTGTGGGTTTAATAGCAGCAGCTAGCTGGATCACAATAT 242     GCTTCCTGGCGCTCTCGAAGAAGGTCCAGATTACGACGGCCGCAAGAACCATAGTCTC 246
GAATGTGCTCCAAGGAGAATGCTGTGTGGGTTTAATAGCAGCAGCTAGCTGGATCACAATAT 300     GCTTCCTGGCGCTCTTGAAGAAGGTCCAGATTACGAGAAGGTCGCAAGAACCATAG---- 206
ATGTGCTGCTCCAAGGAGAATGCTGTGTGGGTTTAATAGCAGCAGCTAGCTGGATCACAATAT 208    GCTTCCCGGCGCTCTCGAAGAAGGTCCAGATTACGAGAAGGTCGCAAGAACCATAG---- 296
******************************************************************          ****** ******** *********************.** *************
```

```
AGCCTTGGGTTCCTTTGAAAGCCCAACAGTTTCCTGGATGTACATCAGGCTGAATGACTG 302        ATAAGCCTACTGCCAATGACTGATCTTGTTCTGTTCAAGATCCCAAATAGACTTAGTCCC 306
AGCCTTGGGTTCCTTTGAAAGCCCAACAGTTTCCTGGATGTACATCAGGCTGAATGACTG 360        ---------------------------------------CCAAATAGACTTAGTCCC 224
AGCCTTGGGTTCCTTTGAAAGCCCAACAGTTTCCTGGATGCACATCAGGCTGAATAACTG 268        ---------------------------------------TCGAATAGACTTAGTCCC 314
**************************************** ************** ****                           *.**************

TCCTTGGTGAATTACTCAGATACCAGAGAGGTATCCCAAACAAACTAAAGAGGGCAGTCT 362        TCCTTAGCGCCACTGCCACCAGATTTATAGACGGATGGATGACTCTCCTTCTGGAAGATC 366
TCCTTGGTGAATTACTCAGATACCAGAGAGGTATCCCAAACAAACTAAAGAGGGCAGTCT 420        TCCTTAGCGCCACTGCCACCAGATTTATAGACGGATGGATGACTCTCCTTCTGAAAGATC 284
TCCTTGGTGAATTACTCAGATACCAGAGAGGTATCCCAAACAAACTAAAGAGGGCGGTCT 328        TCCTTAGCGCCACTGCCACCAGATTTATAGACAGATGGATGACTCTCCTTCTGAAAGATC 374
******************************************************* ****                ********************************.***************** ******

TAAAGGCGTAGGTCTCGGAGCATCTGATACTGATGATACTTCCTCCTGCTGACTCTAGAG 422        TGAAGACTGCTGTATGTATTGACATCAATGCTTACCATATCGGACAAGGAAAATGTCTTG 426
TAAAGGCATAGGTCTCGGAGCATCTGATACTGATGATACTTCCTCCTGCTGACTCTAGAG 480        TGAAGACTGCTGTATGTATTGACATCAATGCTTACCATATCTGACAAGGAAAATGTCTTG 344
TGAAGGCGTAGGTCTCGGAGCATCTGATACTGATGATACTTCCTCCTGCTGACTCTAGAG 388        TGAAGACTGCTGTATGTATTGTCATCAATGCTTACCATATCTGACAAGGAAAATGTCGTG 434
*.***** .*************************************************                ********************* ************** *************** **

CATAGTCCACCATCCCTGTCTTATCAGCATTGTACATGTCAAACGCAGTGATCACAATGC 482        ATGTTCAGGACCGGACTGGAAACATCAGATCGCTCCAACTCCACTCCGATCCTCTTCTTC 486
CATAGTCCACCATCCCTGTCTTATCAGCATTGTACATGTCAAACGCAGTGATCACAATGC 540        ATGTTCAGGACTGGACTAGAAACATCAGATCGCTCCAACTCCACTCCAATCCTCTTCTTC 404
CATAGTCCACCATTCCTGTCTTATCAGCATTGTACATGTCAAACGCAGTGATCACAATGC 448        ATGTTCAGGACTGGACTGGAAACATCAGACCGCTCCAACTCCACTCCAATCCTCTTCTTC 494
************* **********************************************                *********** ***** *********** ***************** ***********

TCTTCACACGATCTTCATCTAGGCTTCCTCCAGCAGCAGCTGATCCTCCGGCTGCTCCAC 542        TCCAAGAACTTCAGAAGTCCTCCAGTGGCACGGATCT 523
TCTTCACACGATCTTCATCTAGGCTTCCTCCAGCAGCAGCTGATCCTCCGGCTGCTCCAC 600        TCCAGGAACTTCAG----------------------- 418
TCTTCACACGATCTTCATCTAGGCTTCCTCCAGCAGCAGCTGATCCTCCGGCTGCTCCAC 508        TCCAGGAACTTCAGAAGTCCTCCAGTGGCACGGATCT 531
************************************************************                ****.**********

TACCACCAGTAGATACGAACGGAAAGAACCATGGCATTC--- 581
TACCACCAGTAGATACGAACGGAAAGAACCATGGCATTCAAT 642
TACCACCAGTAGANNNNNNNGGAA------------------ 532
*************. .  ****
```

```
TOP  : Sycp1-D40                                                         TOP  : MSH4
Bottom: SYCP1                                                            Bottom: Msh4-Ovary
NNNNNNNNNNNNNNNNNNNGTTGGGAGCTCTNNNNNTATGGTCGACCTGCAGGCGGCCGCGA 60      NNNNNNNNNNNNNNNANGNNNNNGCCGCCNTGGCGGCCGCGGGAATTCGATTATAGTAC 60
------------------------------------------------------------              ----------------------------------------------------ATAGTAC 7
                                                                                                                             *******
ATTCACTAGTGATTGTTGCACTGAGCCAGACTCAACTCTTGAAACTTCATTTCCAGGATG 120      TGTTCTGTTGTTCTTCCTCTTGACAGGGTGTGAGTGTAGCTCAGCCTGTTGCTGTCCGCA 120
-------------GTTGCACTGAGCCAGACTCAACTCTTGAAACTTCATTTCCAGGATG 46        TGTTCTGTTGTTCTTCCTCTTGACAGGATGTGAGTGTAGCTGAGTCGTTGCTGTCCTCA 67
             ********************************************              ****************************** *********** ** ********** **

TTCATATCTCCAAGTGTTTCTGCGCGATTATCTTGAAGTTCCTCCTTCACTTGTTCACCC 180      CCCTCGGCTAGAGACTGCTTGACTTGGAAGTGGTAGTTTTCAACATTGGGGTAGAGAGCA 180
TTCATATCTCCAAGTGTTTCTGCGCGATTATCTTGAAGTTCCTCCTTCACTTGTTCACCC 106      CCCTCAGCTAGAGACTGCTCCACTTGGAAGTGGTAGTTTTCAACGTTGGGGTAGAGAGCA 127
********************************************** ***** ********** .***     *****.************ ***********************. ***************

TCCATCAGCTTGCCTTCTAACTTGCCAGCGTAGCCCTTCACCGAGTTACACATCTCCCTC 240      TCGAGGTTGGTGAGTTCCATGAAGTGTGTTGCAAAGAATGTGAATGCTTTGAGACTGAGA 240
TCCATCAGCTTTCCTTCTAACTTGCCAGCGTAACCCTTCACCAAGTTACACATCTCCCGC 166      TCGAGGTTGGTGAGTTCCATGAAGTGTGTTGCAAAGAATGTGAATGCTTTGAGACTGAGG 187
*********** ********************** ******** *************** *              ***********************************************************.

GTTGATACAATTTTCTGCGAGAGTGTCAATTCTGTTTTCTATCTCCTCCTGTAGCCTCACA 300     AGGTATTCACAGATAGCATGGCAGATCCCAACACCTTCGTCGCTACTAGTGCCTCTTCCA 300
GTTGATACAATTTTCTGGGAGATGTCAATTCTGTTTTCTATCTCCTCCTGTAGCCTCACA 226      AGGTATTCACAGATAGCATGGCAGATCCCAACACCTTCGTCACTACTAGTGCCTCTTCCA 247
****************** ***.*************************************              *********************************************.**************

CTAAGGTTCTCATTTTGGAGCTGAAGTTCAAGGAGTGATTTTCTTTGTGATTCAATTGTC 360      AGCTCATCGATAATAACCAGGGATTCATGAGTACAGTTCTGCACAATGTAATTGATTTCT 360
CTAAGGTTCTCATTTTGGAGCTGAAGTTCAAGGAGTGATTTTCTTTGTGATTCAATTGTT 286      AGCTCATCAATGATAACCAGGGATTCATGAGTACAGTTCTGCACAATGTAATTGATTTCT 307
**:*************** ***.**********************************              ********.**.************************************************.

TGTTGTGCATCACTTAATCCTCTTTCCTTCTGCCTCACATCAATCTCCATCTGTACCTTC 420      CTCATCTCTAGTGTAAAGGAGGAAGCGTTGGTTTCGATGTCATCGTCGCACCCGATCCTT 420
TGTTGTGCATCACTCAATCCTCTTTCTTTCTGCCTCACATCAATCTCCATCTGTACCTTC 346      CTCATCTCTAGTGTAAAGGAGGAAGCGTTGGTTTCGATGTCATCATCACACACCCGATCCTT 367
******************************************************              **************************************************.**.************
```

```
*************** ********** ******** ***************** ******
CAGCGGCGTATCTTGTCTGCCTCCTGATGCAAGCGTGAGTGCAAGGCATTCATCTTGTCC 480
CAGCGACGTATCTTGTCTGCTTCCTGATGCAAGCGTGAATGCAAGGCATTCATCTTGTCC 406
*****.*************** **************** .*****************

TGATTCTGTATCTCCAGGGTTTCATTCTCGTGTTGGGCAAGAGAAGTCTGGAACTCTTTG 540
TGATTCTGTATCTCCAGGGTTTCATTCTCGTGTTGGGCTAGAGATGTCTGGAACTCTTTG 466
************************************** :***** :**************

GTCATTTCTGAGGTGTTTTGTAGGAACTGTTCAGATGCTTGAAGGGGCTGCGTCTGGAAG 600
GTCATTTCTGAGGTGTTTTGAAGGAACTGTTCAGATGCTTGAAGGGGCTGCGTCTGGAAG 526
********************* :** *********** ********************

GACTGACCCTTTGAATCGAATTCCCGCGGCCGCCATGGCGGCCGGGAGCATGCGACGTCG 660
GACTGACCCTTTG------------------------------------------------ 539
*************
```

```
GAGAAGACCTGATCACAGATCCTGAATGAAGCGTACTCTGCTGGTACGAAGCAACCTATC 480
GAGAAGACCTGATCACAGATCCTGAATGAAGCGTACTCTGCTGGTACGAAGCAACCTATC 427
************************************************************

TGAGCCATGATCTGAAGCAGCGCTATCTGCTTCAGGTACGTAGACTTGCCCACTCATATTT 540
TGAGCCATGATCTGAAGCAGCGCTATCTGCTTCAGGTACGTAGACTTCCCACTCATATTT 487
*********************************************** ************

GGACCAGTGATGATAAGGAAGTTACCCTCCTCTGATGCATAAACGTTGTTTGGAACTGGC 600
GGACCAGTGATGATAAGGAAGTTACCCTCCTCTGATGCATAAATGTTGTTTGGAACGGGC 547
*******************************************  ************ ***
```

TOP : RAD21
Bottom: Rad21-D40
```
ATGGATAACAGGAAAGACCCAGACCATCGACAATACCATGGAGAAGCCCATGGACCTGGGT 60
ATGGATAACAGGAAAGACCCAGACCATCGACAACACCATGGAGAAGCCCATGGACCTGGGT 60
********************************* *************************

CTGGATGAACCTATCAGGGATGATGGCTTCGGAGCAGAGATGGGCATTGGAAGTGGTATC 120
CTGGATGAACCTATCAGGGATGATGGCTTCGGAGCAGAGATGGGCATTGGAAGTGGTATC 120
************************************************************

CTTGGCAATGAATTCATGGGACCTGAGGATGGCTTGTTTGATGAACAGCCAGAGATCCATT 180
CTTGGCAATGAATTCATGGGACCTGAGGATGGCTTGTTTGATGAACAGCCAGAGATCCAT 180
************************************************************

GCTGGTGAGGTGCCCATTGGAGGTACTGCATCGGGTGAAAGCAGTCCAGAAAGGCCAAAT 240
GCTGGTGAGGTGCCCATTGGAGGTACTGCATCAGGTGAAAGCAGTCCAGAAAGGCCAAAT 240
******************************** .*************************

GAAGAGGAGAAACAAATCAGAGGACAACCAATGGAGCAGGGCGAGGATGTTGCAGAACAA 300
GAAGAGGAGAAACAAATCAGAGGACAACCAATGGAGCAGGGCGAGGATGTTGCAGAACAA 300
************************************************************

CCAGCTGCAGATCAAACAACACTGGTACAAAATGATGAAGATGCATTTGCCCTAGAACCC 360
CCAGCTGCAGATCAAACAACACTGGTACAAAATGATGAAGATGCATTTGCCCTAGAACCC 360
************************************************************

ATTGACATCTCGGCTGGCATGAAAGAAACCAGACAGAGGCGCAAGAGGAAACTGATCGTC 420
ATTGACATCTCGGCTGGCATGAAAGAAACCAGACAGAGGCGCAAGAGGAAACTGATCGTC 420
************************************************************

GACGAGCAGAAGGAGCTCCAGGGCGACATCATCAAATCGCAACTCAAGGACACGTCGGAC 480
GACGAGCAGAAGGAGCTCCAGGGCGACATCATCAAATCGCAACTCAAGGACACGTCGGAC 480
************************************************************

ATCGTGGCCACCCTAGACCTTGCCCCACCCACCAAGAAACTCATGCTGTGGAAGGAGACT 540
ATCGTGGCCACCCTAGACCTTGCCCCACCCACCAAGAAACTCATGCTGTGGAAGGGAGACT 540
*********************************************************

GGTGGTGCCGAGAAGCTCTTCTGCTTGCCAGCACGGCAACACACGTCAATATTTATTCTA 600
GGTGGTGCCGAGAAGCTCTTCTGCTTGCCAGCACGGCAACACACGTCAATATTCATTCTA 600
**************************************************** *******
```

TOP : RAD51
Middle: Rad51-Ovary
Bottom: Rad51-D40
```
ATGGCAATGCAAATGCACAATGCTGAGCAGGAAGTTTCTGAGGAGTTTGGTCCACTCGGT 60
ATGGCAATGCAAATGCACAATGCTGAGCAGGAAGTTTCTGAGGAGTTTGGTCCACTCGGT 60
ATGGCAATGCAAATGTACAATGCTGAGCAGGAAGTTTCTGAGGAGTTTGGTCCACTCGGT 60
*************** *********************************************

ATCAGTAGGCTTGAGGCTAGTGGAATAAGCTCTAATGATGTGAAGAAGCTGGAGGAAGCA 120
ATCAGTAGGCTTGAGGCTAGTGGAATAAGCTCTAATGATGTGAAGAAGCTGGAGGAAGCA 120
ATCAGTAGGCTTGAGGCTAGTGGAATAAGCTCTAATGATGTGAAGAAGCTGGAGGAAGCA 120
************************************************************

GGTATGCACACTGTGGAGTCTGTGGCTTACTCTACCAAGAAAGAACTGTGTGCTGTCAAG 180
GGTATGCACACTGTGGAGTCTGTGGCTTACTCTACCAAGAAAGAACTGTGTGCTGTCAAG 180
GGTATGCACACTGTGGAGTCTGTGGCTTACTCTACCAAGAAAGAACTGTGTGCTGTCAAG 180
************************************************************

GGTATCAGTGAGGCTAAGGCAGATAAGATCCTGACGGAGGCCCAGAAGCTTGTTCCTATG 240
GGTATCAGTGAGGCTAAGGCAGATAAGATCCTGAATGAGGCCCAGAAGCTTGTTCCGATG 240
GGTATCAGTGAGGCTAAGGCAGATAAAATCCTGACGGAGGCCCAGAAGCTTGTTCCTATG 240
************************* .*******. *****************  ***

GGCTTCACCACGGCTACTCAGTTCCACCAACAGAGATCAGAGATCATACAGGTCACAACT 300
GGCTTCACCACGGCTACTCAGTTCCACCAACAGAGATCAGGGATCATACAGGTCACAACT 300
GGCTTCACCACGGCTACTCAGTTCCACCAACAGAGATCAGAGATCGTACAGGTCACAACT 300
****************************************.**** .************

GGCTCCAAGGAGCTGGACAAACTTCTACAAGGTGGTATTGAGACTGGTTCCATCACAGAA 360
GGCTCCAAGGAACTGGACAAACTTCTACAAGGTGGTATTGAGACTGGTTCTATCACAGAA 360
GGCTCCAAGGAGCTGGACAAACTTCTACAAGGTGGTATTGAGACTGGTTCTATCACAGAA 360
***********.************************************* *********

ATCTTTGGAGAGTTCAGAACTGGAAAGACACAACTCTGCCATACTATGGCAGTGACTTGT 420
ATCTTTGGAGAGTTCAGAACTGGAAAGACACAACTCTGCCATACTATGGCAGTGACTTGT 420
ATCTTTGGAGAGTTCAGAACTGGAAAGACACAACTCTGCCATACTATGGCAGTGACTTGT 420
************************************************************

CAGCTACCTATTGACAATGGTGGAGGAGAAGGGAAATGTCTCTACATAGACACAGAGGGT 480
CAGCTACCTATTGACAATGGTGGAGGAGAAGGGAAATGTCTGTACATAGACACAGAGGGT 480
************************************* **********************
```

```
**********************************************************  ******              CAGCTACCTATTGACAATGGTGGAGGAGAAGGTAAATGTCTCTACATAGACACAGAGGGT 480
                                                                              ************************************ ******** ******************
TCTATTTACAGACGGAATCTCACCGCTGACATTCCAAAGGATCTCAAGATTGTCAGAGAT 660
TCTATTTACAGACGGAATCTCACCGCTGACATTCCAAAGGATCTCAAGATTGTCAGAGAT 660           ACCTTCCGACCTGAGAGATTGATTGCTGTAGCAGACAGATACAATCTGTCAGGCAGTGAT 540
**********************************************************               ACCTTCCGACCTGAGAGATTGATTGCTGTAGCAGACAGATACAATCTGTCAGGCAGTGAT 540
                                                                              ACCTTCCGACCTGGGAGATTGATTGCCGTAGCAGACAGATACAATCTGTCAGGCAGTGAT 540
GAAGACTTTGAGCTTGAGGAGCCTGAAGTACAGCGAAGAGTAGAGCATCATGACTTCACC 720                 ************* ************  **********************************
GAAGACTTTGAGCTTGAGGAGCCTGAAGTACAGCGAAGAGTAGAGCATCATGACTTCACC 720
**********************************************************               GTGTTGGACAACGTAGCGTATGCGAGAGCTCACAACTCAGATCATCAGTCACAACTCCTT 600
                                                                              GTGTTGGACAACGTAGCGTATGCGAGAGCTCACAACTCAGATCATCAGTCACAACTCCTT 600
TTGGAAGCCTCCAGAGACCAGACATCATTGTCCATTGACGAACCCTCCTTGATCGAGGAC 780              GTGTTGGACAACGTAGCGTATGCGAGAGCTCACAACTCAGATCATCAGTCACAACTCCTT 600
TTGGAAGCCTCCAGAGACCAGACATCATTGTCCATTGACGAACCCTCCAGGATCGAGGAC 780                 **********************************************************
*********************************************** : **********
                                                                              CTTCAGGCTTCAGCAATGATGGCAGAATCAAGGTATGCTTTGCTGATTGTAGACAGTGCC 660
CAGCCCAGCACCTCAGCCCACACCCGCTCCCCCTACTCCAGCCCACATCCCCATGTCCAGG 840             CTTCAGGCTTCAGCAATGATGGCAGAATCAAGGTATGCTTTGCTGATCGTAGACAGTGCC 660
CAGCCCAGCACCTCAGCCCACACCCGCTCCCCCTACTCCAGCCCACATCCCCATGTCCAGG 840             CTTCAGGCTTCAGCAATGATGGCAGAATCAAGGTATGCTTTGCTGATCGTAGACAGTGCC 660
**********************************************************               ************************************* ******* ************
AGATCCTCCTCATCGGACGGTGGGGACTACGGCGGTGACCTCTTTGCCGCTGACTACGAC 900             ACAGCACTTTATCGCACAGACTACTCCGGGCGTGGTGAGCTAGCCTCAAGGCAGATGCAT 720
AGATCCTCCTCATCGGACGGTGGGGACTACGGCGGTGACCTCTTTGCCGCTGACTACGAC 900             ACAGCACTTTATCGCACAGACTACTCCAGGCGTGGTGAGCTAGCCTCAAGGCAGATGCAT 720
**********************************************************               ACAGCACTTTATCGCACAGACTACTCCGGGCGTGGTGAGCTAGCCTCAAGGCAGATGCAT 720
                                                                              *************************** ** ******************************
GATGATTACGATATTCCGTCCGTCGCGCCGCAGTCCGTCGGACCACAGGCCGTTGAAGAA 960
GATGATTACGATATTCCGTCCGTCGCGCCGCAGTCCGTCGGACCACAGGCCGTTGAAGAA 960             TTGGGGAGGTTTTTAAGAACACTGCTCAGACTTGCTGATGAGTATGGTGTTGCTGTGGTG 780
**********************************************************               TTGGGGAAGGTTTTTAAGAACACTGCTCAGACTTGCTGACGAGTATGGTGTTGCTGTGGTG 780
                                                                              TTGGGGAAGGTTTTTAAGAGCACTGCTCAGACTTGCTGATGAGTATGGTGTTGCTGTGGTG 780
CCAGAGGAGGAGGAAGAGCTTCAAGAGGGTGAAGACCAAGAAGCACACGAGGAGAGGAAG 1020                ***** ************  ******************** ***************
CCAGAGGAGGAGGAAGAGCTTCAAGAGGGTGAAGACCAAGAAGCACACGAGGAGAGGAAG 1020
**********************************************************               ATCACTAACCAGGTTGTCGCTCAAGTAGATGGTGCAGCGATGTTCACTTCAGATCCTAAG 840
                                                                              ATCACTAATCAGGTTGTCGCTCAAGTAGATGGTGCAGCGATGTTCACTTCAGATCCTAAG 840
CTGAACAAGAGAGCACAAATCATGTTGACAGCAATCAATACCAGACTACAGACCTCATCG 1080            ATCACTAATCAGGTTGTCGCTCAAGTAGATGGTGCAGCGATGTTCACTTCAGATCCTAAG 840
CTGAACAAGAGAGCACAAATCATGTTGACAGCAATCAATACCAGACTACAGACCTCATCG 1080               ********  ************************************************
**********************************************************
                                                                              AAACCCATAGGAGGTCATATCATGGCTCATGCTTCCACTACAAGGCTATACCTGAGGAAA 900
GAAGTATCGTTCAAGCATGATGTCACAGCGAGACTTTACAGGAAGCAAGCTGCAGCCAAA 1140            AAACCCATAGGGGGTCATATCATGGCTCATGCTTCCACTACAAGGCTATACCTGAGGAAA 900
GAAGTATCGTTCAAGCATGATGTCACAGCGAGACTGTACAGGAAGCAAGCTGCAGCCAAA 1140            AAACCCATAGGAGGTCATATCATGGCTCATGCTTCCACTACAAGGCTATACCTGAGGAAG 900
*********************************** ********************               ***********  ***********************************************  .
TTCTACACCCTTCTTGTCTTGAAGAAGCAACAAGCCATCGATGTCTTCCAGAACGTCACG 1200            GGTCGAGGTGAGACCAGGATATGTAAGATCTATGATTCCCCCTGCCTGCCTGAAGCAGAA 960
TTCTACACCCTTCTTGTCTTGAAGAAGCAACAAGCCATCGATGTCTTCCAGAACGTCACG 1200            GGTCGAGGTGAGACCAGGATATGTAAGATCTATGATTCCCCCTGCCTGCCTGAAGCAGAA 960
**********************************************************               GGTCGGGGTGAGACCAGGATATGTAAGATCTATGATTCCCCCTGCCTGCCTGAAGCAGAA 960
                                                                              ***** ****************************************************
TATGGAGACATCACCAT 1217
TATGGAGACATCACCAT 1217                                                      GCCATGTTTGCCATCAATCCCGATGGTGTTGGTGATGCCAAGGACTGA 1008
*****************                                                          GCCATGTTTGCCATCAATCCCGATGGTGTTGGTGATGCCAAGGACT-- 1006
                                                                              GCCATGTTTGCCATCAATCCTGATGGTGTTGGTGATGCCAAGGACT-- 1006
                                                                              ******************** ************************
```

*
 Top indicates the gene sequence annotated in SpBase.org, and Middle/Bottom indicates the cloned sequence obtained either from Ovary/Day40 (D40) cDNAs.

Table 3

Peptide sequence alignments of each meiotic protein between sea urchin and human, mouse or *C.elegans*.

```
Top  : Human DMC1
Bottom: S.purpuratus DMC1
EDQVVAE--EPGFQDEEESLFQDIDLLQKHGINVADIKKLKSVGICTIKGIQMTTRRALC 60
+DQVV E  +  +D+EES FQDID+LQ HGI                GI MTTR+ +C
QDQVVDETIDGTLEDDEESFFQDIDMLQNHGI----------------GIIMTTRKRMC 44

NVKGLSEAKVDKIKEAANKLIEPGFLTAFEYSEKRKMVFHITTGSQEFDKLLGGGIESMA 120
++KG+SEAK++KIKEAA+KL + GF TA EYS KR+ VF ITTGS E DKLLGGGIESMA
DIKGISEAKMEKIKEAASKLEDHGFTTALEYSVKRRNVFRITTGSTELDKLLGGGIESMA 104

ITEAFGEFRTGKTQLSHTLCVTAQLPGAGGYPGGKIIFIDTENTFRPDRLRDIADRFNVD 180
ITEAFGEFRTGKTQLSHTLCV  QLPG+ GYPGGK+IFIDTENTFRPDRLRDIADRFN+D
ITEAFGEFRTGKTQLSHTLCVCTQLPGSNGYPGGKVIFIDTENTFRPDRLRDIADRFNLD 164

HDAVLDNVLYARAYTSEHQMELLDYVAAKFHEEAGIFKLLIIDSIMALFRVDFSGRGELA 240
H A+LDNVLYARAYTSEHQ ELLDY A KFHEE G+FKLLIIDSIMALFRVDF+GRGELA
HGAMLDNVLYARAYTSEHQFELLDYAAGKFHEEPGVFKLLIIDSIMALFRVDFTGRGELA 224

ERQQKLAQMLSRLQKISEE 259
+RQQKLAQMLS+LQKISE+
DRQQKLAQMLSKLQKISED 243
```

```
Top  : Mouse SYCP1
Bottom: S.purpuratus SYCP1
IVAVVEGRGLARGEIGMASIDLKSPQIMLSQFADNTTYAKVITKLQVLSPLEIIMSNTAC 267
++A+VEGRG+A+GEIGMASIDLK P+++LSQF+D+ TY KV TKLQVL P+EIIMS+TAC
MIAIVEGRGIAQGEIGMASIDLKQPELILSQFSDSQTYVKVTTKLQVLQPIEIIMSHTAC 60

VVGNSTKLFTLITENFKNVNFTTVQRKYFNETKGLEYIEQLCIAEFSSVLMEVQSRYYCX 327
G  TKLF LI++NF+ ++ TT+QRKYFNETKGL  ++QLC+A+FSSV ME+ S+YYC
EGGAMTKLFKLISDNFQGLDVTTIQRKYFNETKGLSVVKQLCVADFSSVEMEITSKYYCL 120

XXXXXXXKYVEFIQNSVYAPKSLKIYFQGSEQTAMIDSSSAQNLELLVNNQDYRSNHTLF 387
KYVEFIQNSVYAP SLK+ F+GSEQ+A+ID+ +A+NLEL+ N +D +S+HTLF
AAAAALLKYVEFIQNSVYAPNSLKVVFRGSEQSAVIDAVTARNLELVQNRKDPKSDHTLF 180

GVLNYTKTAGGSRRLRSNILEPLVDVETISMRLDCVQELLQDEELFFGLQSVISRFLDTE 447
GVLNYTKT GG+R +RSNIL+P  D ETI +RLD V EL ++EELF+ LQSV+SRFLD +
GVLNYTKTMGGARLMRSNILQPPFDEETIKLRLDVVAELTENEELFYNLQSVLSRFLDVD 240

QLLSVLVQIPKQDTVNAAESKITNLIYLKHTLELVEPLKVTLKNCSTPLLRAYYGSLEDH 507
LLS+ VQIPK+++V ++E KITN+I+LKHTLELVEPL V L++  + L +AY+ +L+D
HLLSLCVQIPKEESVKSSEQKITNVIHLKHTLELVEPLHVALQSAQSKLPQAYHKTLQDT 300

RFGLILDKIKTVINDDARYMKGCLNMRTQKCYAVRSNISEFLDIARRTYTEIVDDIAGMI 567
RF  IL+KI TVIND+ RY KG LNMRTQKC+AV+  I+  LD+ARRTYTE+VDDI+ MI
RFQQILEKIATVINDETRYQKGTLNMRTQKCFAVKPEINGLLDVARRTYTELVDDISEMI 360

AQLAEKYSLPLRTSFSSSRGFFIQM-TTDCAALSSDQLPSEFIKISKVKNSYSFTSADLI 626
QL++++ L LRTS+S++RGFFIQ+     ++  + LP FIK+ K KN+ SFT+ DLI
KQLSDEHGLMLRTSYSTARGFFIQLYCKGKYSIPVESLPPVFIKVVKAKNTLSFTTTDLI 420
```

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Top  : Mouse MSH4
Bottom: S.purpuratus MSH4
IVAVVEGRGLARGEIGMASIDLKSPQIMLSQFADNTTYAKVITKLQVLSPLEIIMSNTAC 267
++A+VEGRG+A+GEIGMASIDLK P+++LSQF+D+ TY KV TKLQVL P+EIIMS+TAC
MIAIVEGRGIAQGEIGMASIDLKQPELILSQFSDSQTYVKVTTKLQVLQPIEIIMSHTAC 60

VVGNSTKLFTLITENFKNVNFTTVQRKYFNETKGLEYIEQLCIAEFSSVLMEVQSRYYCX 327
G  TKLF LI++NF+ ++ TT+QRKYFNETKGL  ++QLC+A+FSSV ME+ S+YYC
EGGAMTKLFKLISDNFQGLDVTTIQRKYFNETKGLSVVKQLCVADFSSVEMEITSKYYCL 120

XXXXXXXXKYVEFIQNSVYAPKSLKIYFQGSEQTAMIDSSSAQNLELLVNNQDYRSNHTLF 387
KYVEFIQNSVYAP SLK+ F+GSEQ+A+ID+ +A+NLEL+ N +D +S+HTLF
AAAAALLKYVEFIQNSVYAPNSLKVVFRGSEQSAVIDAVTARNLELVQNRKDPKSDHTLF 180

GVLNYTKTAGGSRRLRSNILEPLVDVETISMRLDCVQELLQDEELFFGLQSVISRFLDTE 447
GVLNYTKT GG+R +RSNIL+P  D ETI +RLD V EL ++EELF+ LQSV+SRFLD +
GVLNYTKTMGGARLMRSNILQPPFDEETIKLRLDVVAELTENEELFYNLQSVLSRFLDVD 240

QLLSVLVQIPKQDTVNAAESKITNLIYLKHTLELVEPLKVTLKNCSTPLLRAYYGSLEDH 507
LLS+ VQIPK+++V ++E KITN+I+LKHTLELVEPL V L++  + L +AY+ +L+D
HLLSLCVQIPKEESVKSSEQKITNVIHLKHTLELVEPLHVALQSAQSKLPQAYHKTLQDT 300

RFGLILDKIKTVINDDARYMKGCLNMRTQKCYAVRSNISEFLDIARRTYTEIVDDIAGMI 567
RF  IL+KI TVIND+ RY KG LNMRTQKC+AV+  I+  LD+ARRTYTE+VDDI+ MI
RFQQILEKIATVINDETRYQKGTLNMRTQKCFAVKPEINGLLDVARRTYTELVDDISEMI 360

AQLAEKYSLPLRTSFSSSRGFFIQM-TTDCAALSSDQLPSEFIKISKVKNSYSFTSADLI 626
QL++++ L LRTS+S++RGFFIQ+     ++  + LP FIK+ K KN+ SFT+ DLI
KQLSDEHGLMLRTSYSTARGFFIQLYCKGKYSIPVESLPPVFIKVVKAKNTLSFTTTDLI 420
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KMNERCQESLREIYHMTYMIVCKLLSEIYEHIHCLYKLSDTVSMLDMLLSFAHACTLSDY 686
KMN+R +ESL EIY MT ++V +LL++I EH+ CLYKL++ VS++DML+SFAHACTLSDY
KMNDRVRESLNEIYLMTNVVVSQLLNDIREHVGCLYKLAECVSVIDMLVSFAHACTLSDY 480

VRPEFTDTLAIKQGWHPILEKISAEKPVANNTYITEGSNVLIITGPNMSGKSTYLKQIAL 746
VRP+FTDTLAIKQ  HPIL+KIS + PV NN Y +E  N LIITGPNMSGKSTYLKQIAL
VRPDFTDTLAIKQARHPILDKISFDPPVPNNIYASEEGNFLIITGPNMSGKSTYLKQIAL 540

CQIMAQIGSYVPAEYASFRIAAQIFTRISTDDDIETNSSTFMKEMKEIAYILHNANDKSL 806
QIMAQIG +VPAEYASFRI  Q+F+RI  DDDIETN+S+F  EM+EI YI+ N   +SL
LQIMAQIGCFVPAEYASFRICDQVFSRIGCDDDIETNASSFTLEMREINYIVQNCTHESL 600

ILIDELGRGTNTEEGIGISYAVCEHLLSIKAFTLFTTHFLELCHLDALYLNVENMHFEVQ 866
++IDELGRGT+++EG+GI +A+CE+LLS +KAFT F THF+EL +LDALY NVEN HF+V+
VIIDELGRGTSSDEGVGICHAICEYLLSLKAFTFFATHFMELTNLDALYPNVENYHFQVE 660

HVKNTSRNKDAILYTYKLSRGLTEEKNYGLKAAEASSLPSSIVLDARDITTQITRQ 922
+ + + YT+ LSRG T E+ YG+K AE S+LP SIV +A+ ++ ++  Q
QSLAEGEDSNRLSYTHILSRGRTTEQYYGIKLAEISALPPSIVREAKALSQKLAHQ 716
```

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KMNERCQESLREIYHMTYMIVCKLLSEIYEHIHCLYKLSDTVSMLDMLLSFAHACTLSDY 686
KMN+R +ESL EIY MT ++V +LL++I EH+ CLYKL++ VS++DML+SFAHACTLSDY
KMNDRVRESLNEIYLMTNVVVSQLLNDIREHVGCLYKLAECVSVIDMLVSFAHACTLSDY 480

VRPEFTDTLAIKQGWHPILEKISAEKPVANNTYITEGSNVLIITGPNMSGKSTYLKQIAL 746
VRP+FTDTLAIKQ  HPIL+KIS + PV NN Y +E  N LIITGPNMSGKSTYLKQIAL
VRPDFTDTLAIKQARHPILDKISFDPPVPNNIYASEEGNFLIITGPNMSGKSTYLKQIAL 540

CQIMAQIGSYVPAEYASFRIAAQIFTRISTDDDIETNSSTFMKEMKEIAYILHNANDKSL 806
QIMAQIG +VPAEYASFRI  Q+F+RI  DDDIETN+S+F  EM+EI YI+ N   +SL
LQIMAQIGCFVPAEYASFRICDQVFSRIGCDDDIETNASSFTLEMREINYIVQNCTHESL 600

ILIDELGRGTNTEEGIGISYAVCEHLLSIKAFTLFTTHFLELCHLDALYLNVENMHFEVQ 866
++IDELGRGT+++EG+GI +A+CE+LLS +KAFT F THF+EL +LDALY NVEN HF+V+
VIIDELGRGTSSDEGVGICHAICEYLLSLKAFTFFATHFMELTNLDALYPNVENYHFQVE 660

HVKNTSRNKDAILYTYKLSRGLTEEKNYGLKAAEASSLPSSIVLDARDITTQITRQ 922
+ + + YT+ LSRG T E+ YG+K AE S+LP SIV +A+ ++ ++  Q
QSLAEGEDSNRLSYTHILSRGRTTEQYYGIKLAEISALPPSIVREAKALSQKLAHQ 716
```

Top : *C.elegans* RAD-51
Bottom: *S.purpuratus* RAD51
```
IDKLESSGISSGDISKLKEAGYYTYESLAFTTRRELRNVKGISDQKAEKIMKEAMKFVQM 138
I +LE+SGISS D+ KL+EAG +T ES+A++T++EL  VKGIS+ KA+KI+ EA K V M
ISRLEASGISSNDVKKLEEAGMHTVESVAYSTKKELCAVKGISEAKADKILTEAQKLVPM 80

GFTTGAEVHVKRSQLVQIRTGSASLDRLLGGGIETGSITEVYGEYRTGKTQLCHSLAVLC 198
GFTT  + H +RS+++Q+ TGS  LD+LL GGIETGSITE++GE+RTGKTQLCH++AV C
GFTTATQFHQQRSEIIQVTTGSKELDKLLQGGIETGSITEIFGEFRTGKTQLCHTMAVTC 140

QLPIDMGGGEGKCMYIDTNATFRPERIIAIAQRYNMDSAHVLENIAVARAYNSEHLMALI 258
QLPID GGGEGKC+YIDT  TFRPER+IA+A RYN+  + VL+N+A ARA+NS+H   L+
QLPIDNGGGEGKCLYIDTEGTFRPERLIAVADRYNLSGSDVLDNVAYARAHNSDHQSQLL 200

IRAGAMMSESRYAVVIVDCATAHFRNEYTGRGDLAERQMKLSAFLKCLAKLADEYGVAVI 318
++A AMM+ESRYA++IVD ATA +R +Y+GRG+LA RQM L  FL+ L +LADEYGVAV+
LQASAMMAESRYALLIVDSATALYRTDYSGRGELASRQMHLGRFLRTLLRLADEYGVAVV 260

ITNQVVAQVDGGASMFQADAKKPIGGHIIAHMSSTTRLYLRKGKGENRVAKMVQSPNLPEA 378
ITNQVVAQVD GA+MF +D KKPIGGHI+AH STTRLYLRKG+GE R+ K+  SP LPEA
ITNQVVAQVD-GAAMFTSDPKKPIGGHIMAHASTTRLYLRKGRGETRICKIYDSPCLPEA 319

EATYSITNHGIEDARE 394
EA ++I  G+ DA++
EAMFAINPDGVGDAKD 335
```

Top : *C.elegans* MSH-5
Bottom: *S.purpuratus* MSH5
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IGIMVFLSHIGSFVPARHAKIGIVDRIVTRMFTVDSVLDGMSTFAKDVEQVALALRKATG 711
+G++ F++ IGSFVPA  A IG++D I TR+ T +SV  G+STF  D+ Q++ A+R AT
GLIAFMALIGSFVPAEKASIGMLDGIYTRVHTQESVSVGLSTFMIDLNQLSAAVRDATK 60

NSLVIIDEFGKGTMTEVGLSLLASVMTYWMNRGADRCPHIFLSSHFHAL--PNYIPLETN 769
NSLVI+DEFG+ T T  GLSLL + + +W  +G+D CP++ +S+HFHA+    +P
NSLVIVDEFGRSTDTVDGLSLLVACIKHWEGKGSD-CPNLMVSTHFHAIVREGLLPASQQ 119

IATFLTFTVLREAGGKIKYLFRMTPGLVDCSFALSVAKEEGIPPPVIGRACRIYKALKAG 829
+ ++ T  VL E  G++ +L +T G  S A  +A  G+P  ++ R  ++ K L+
L-SYQTMEVL-ENDGELVFLYELTDGHANYSHASHIALTAGLPEELVKRGTKVSKLLREN 177

TLLKEI---KAEVSNDNEKQLVE---DMDVVLADEDGFM 862
+ +   +E  D K+++  D+D+  D GF+
QPILRVDSSSSETQFDRYKEILAKFLDLDLDFDDLTGFL 216
```