MEIOSIS AND RECOMBINATION

IBS 605 -- Fall 2009

color handouts at: **http://lectures.paralog.com/IBS605-Pierce.pdf**

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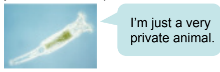
What is meiosis?

- reduction in chromosome complement from diploid to haploid

- generate diversity by random segregation of homologs
- generate diversity by recombination between homologs
- required for sexual reproduction (all multicellular organisms)



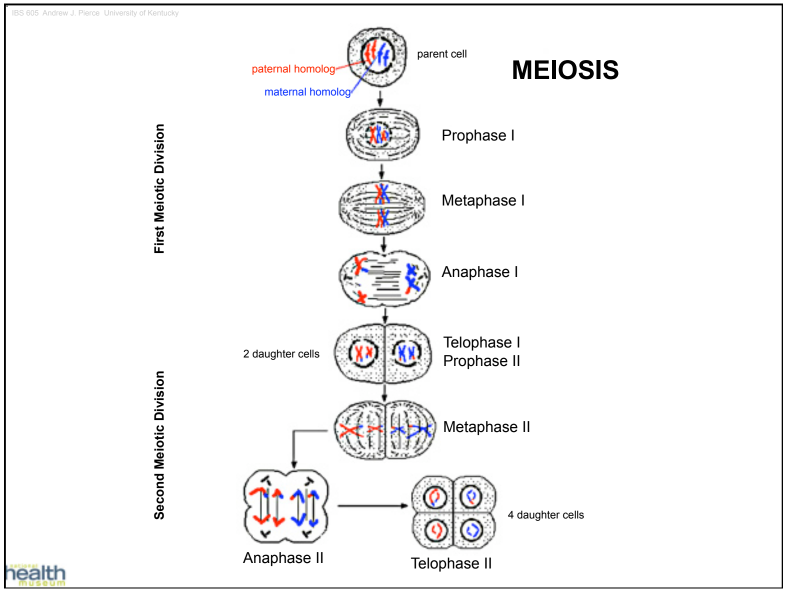
bdelloid rotifer *Philodina roseola*

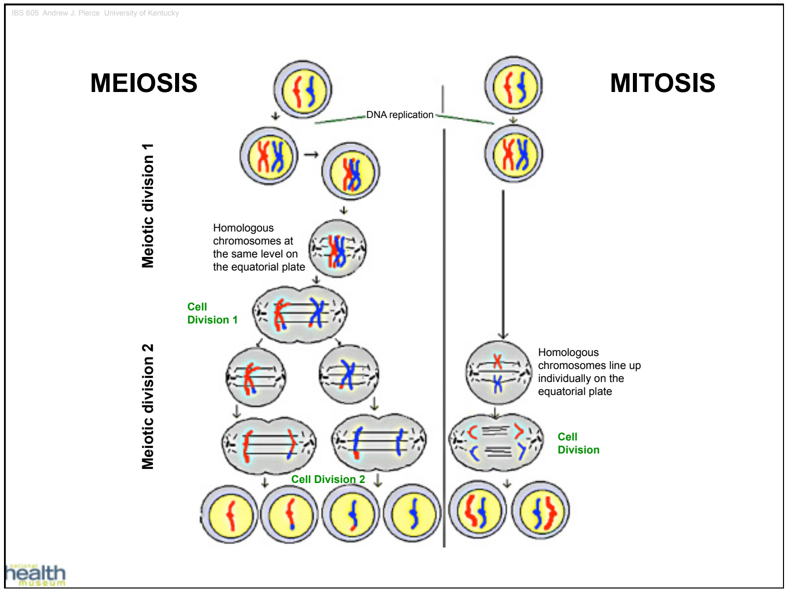
- one round of DNA replication followed by two rounds of cell division

- separate homologs in first division (meiosis I)
  - *How does the cell know which chromosomes are homologs?*

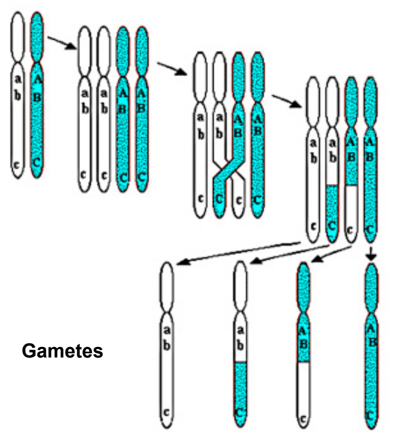
- separate sisters in second division (meiosis II)
  - *similar to mitosis*

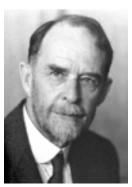
- Cytogenetics vs Molecular Genetics


MEIOSIS
parent cell
paternal homolog
maternal homolog
First Meiotic Division
Prophase I
Metaphase I
Anaphase I
2 daughter cells
Telophase I
Prophase II
Second Meiotic Division
Metaphase II
Anaphase II
Telophase II
4 daughter cells


MEIOSIS
MITOSIS
DNA replication
Meiotic division 1
Homologous chromosomes at the same level on the equatorial plate
Cell Division 1
Meiotic division 2
Cell Division 2
Homologous chromosomes line up individually on the equatorial plate
Cell Division

Crossing-over and Recombination During Meiosis





Gametes

Adapted from: Morgan T.H., Sturtevant A.H., Muller H.J., and Bridges C.B., "The Mechanism of Mendelian Heredity", 1915.

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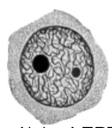
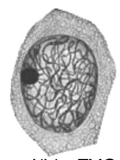
http://www.pbs.org/wgbh/nova/miracle/divide.html

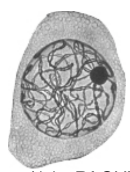
Stages of Meiosis -- Prophase I



Prophase I(a): LEPTOTENE
Chromosomes first become visible as fine threads and have not yet associated in pairs.
(Gk. leptos, thin; taenia, ribbon.)



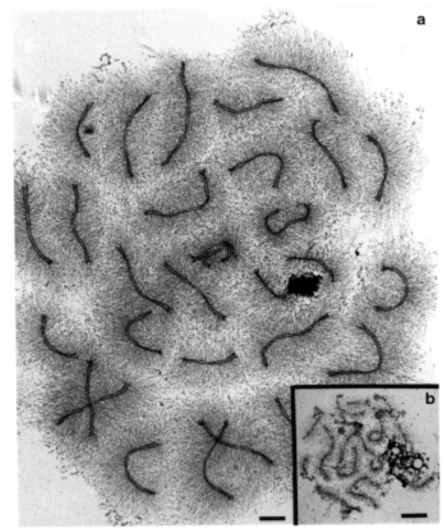
Prophase I(b): ZYGOTENE
Homologous chromosomes are associating side by side.
(Gk. zygon, yoke; taenia, ribbon.)



Prophase I(c): PACHYTENE
Homologous chromosomes are associated throughout their length.
(Gk. pachys, thick; taenia, ribon.)

Birgid Schlindwein's Hypermedia Glossary Of Genetic Terms

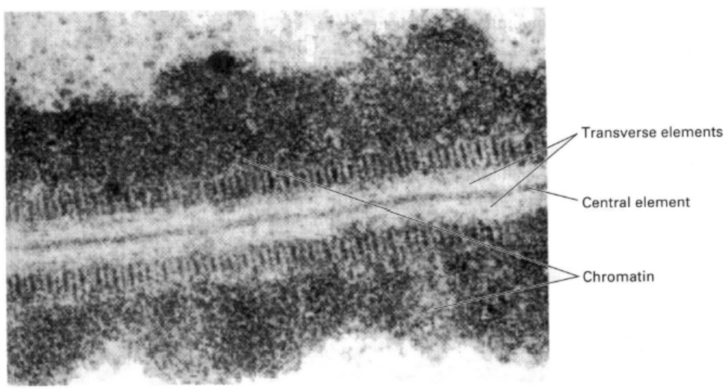
Full Synapsis At Pachytene



Spread pachytene nuclei from the moth *Hyalophora columbia* (*a*) and from the yeast *Saccharomyces cerevisiae* (*b*) with hyponically decondensed chromatin loops extending from the SCs (strongly stained double lines). Both nuclei are at the same magnification and show clearly the difference in loop sizes between the two species (Bars = 2 m).

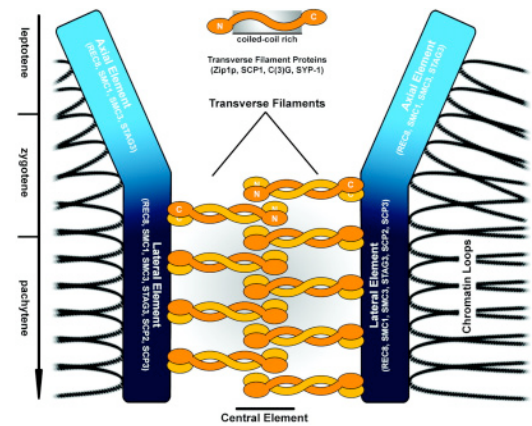
Zickler D, Kleckner N. **The leptotene-zygotene transition of meiosis.** Annu Rev Genet. 1998;32:619-97.
Data from Moens PB, Pearlman RE. 1988. Chromatin organization at meiosis.*BioEssays* 9:151-53

Synaptonemal Complex



Biol139, University of Waterloo

SYNAPTONEMAL COMPLEX COMPONENTS



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Stages of Meiosis -- Prophase I (cont'd)

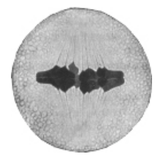
Prophase I(d): DIPLOTENE

The two chromosomes making up each homologous pair have separated from one another except at nodes (chiasmata) distributed along their length.
The successive loops between the chiasmata all lie in one plane.
(Gk. diploos, double; taenia, ribbon.)

Prophase I(e): DIAKINESIS

The chromosomes are well separated from one another.
This stage is recognized by the highly condensed condition of the chromosomes, the homologous pair of which are held together by chiasmata.
(Gk. kinesis, movement; dia, apart)

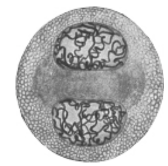
The Remaining Stages of Meiosis



Metaphase I

Anaphase I

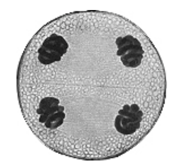
Telophase I



Interkinesis / Prophase II

Metaphase II

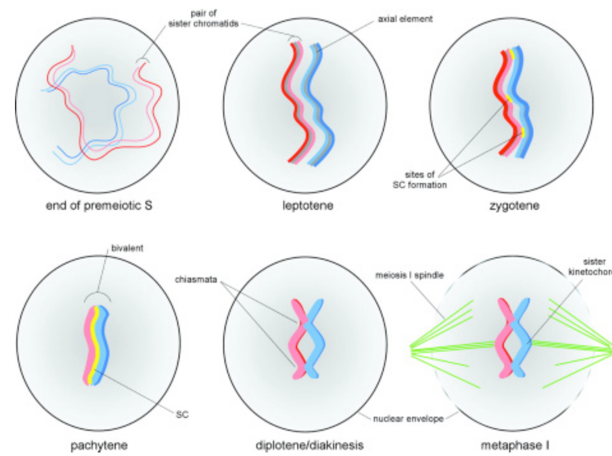
Anaphase II



Telophase II

http://www.contexo.info/DNA_Basics/images/meiosis_movie.mov

PROPHASE I -- MOLECULAR DETAILS



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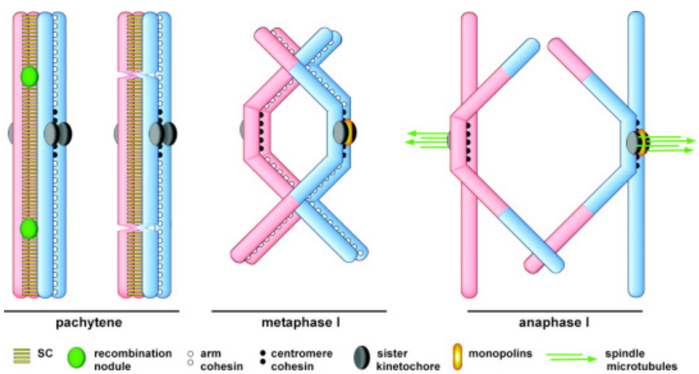
Cross-overs Hold Homologs Together



Cohesins remain attached at centromeres

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Limiting the Number of Chiasmata

Multiple chiasmata are commonly found (in humans the average number of chiasmata per tetrad is just over two). In this photomicrograph (courtesy of Prof. Bernard John), a tetrad of the grasshopper **Chorthippus parallelus** shows 5 chiasmata.

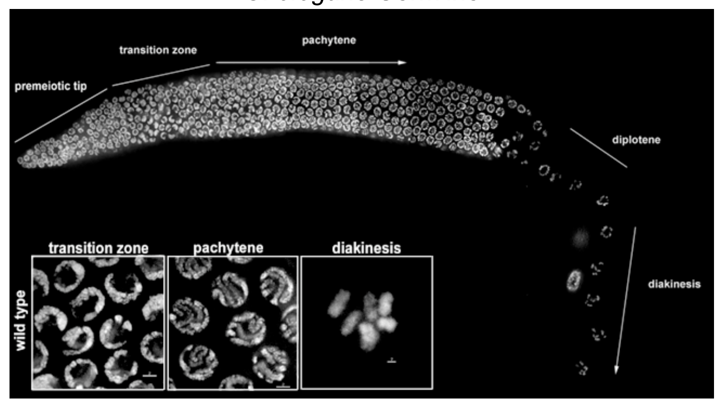
- All chromosome homolog pairs have at least one chiasma (Gk. chiasma; cross)
- Chiasmata are sites of genetic exchange (recombination, crossing-over)
- Chiasmata demonstrate *positive interference*

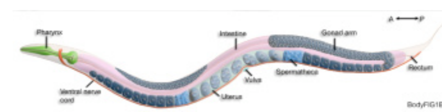
http://biology-pages.info

http://www.wormatlas.org/handbook/fig.s/BIRDSEYEMOVIE.qt

http://www.bio.unc.edu/faculty/goldstein/lab/crawl.mov

C. elegans Germline





Dr. Monica Colaiacovo
Harvard Medical School
Department of Genetics

http://www.wormatlas.org/handbook/bodyshape.htm

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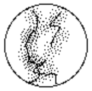
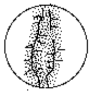
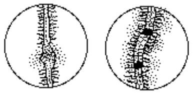
Meiotic Prophase I



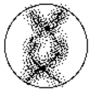
Leptotene
Axial elements begin to decorate chromosomal fibers



Early Zygotene
Axial elements approach one another, becoming lateral elements



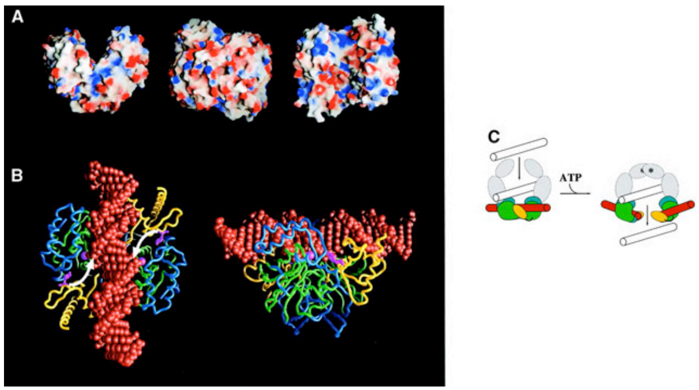
Late Zygotene / Pachytene
Mature synaptonemal complexes consist of a pair of parallel lateral elements flanking a central element. The elements are connected by transverse fibers.



Diplotene
Fewer nodules are present after desynapsis

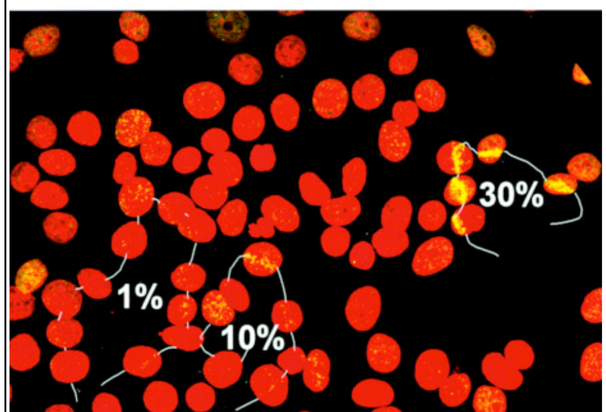
Urich Melcher, Oklahoma State University

Spo11 Makes Chromosomal Double-strand Breaks



Structure and function of an archaeal topoisomerase VI subunit with homology to the meiotic recombination factor Spo11
Matthew D. Nichols, Kristen DeAngelis, James L. Keck and James M. Berger *The EMBO Journal* (1999) **18,** 6177–6188

Double-strand Breaks Trigger Phosphorylation of Histone H2AX



γH2AX foci seen after laser-directed DNA double-strand breaks in MCF7 cells.
UVA light was delivered by a 390-nm laser. The white lines trace the path of the laser as guided with a joystick. The percentages refer to the relative laser energy used in each transit. Cells were incubated with Hoechst dye 33258.

J Cell Biol. 1999 Sep 6;146(5):905-16.
Megabase chromatin domains involved in DNA double-strand breaks in vivo.
Rogakou EP, Boon C, Redon C, Bonner WM.

bonus extra reading:

γ-H2AX illuminates meiosis
Neil Hunter, G. Valentin Borner, Michael Lichten, Nancy Kleckner
Nature Genetics: 27: 236-238 (2001)

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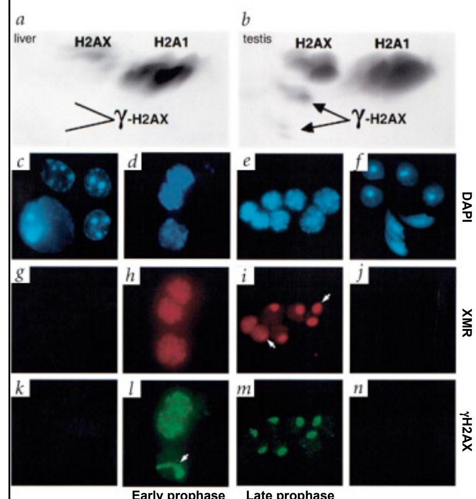
DSBs coincide with early meiotic prophase I

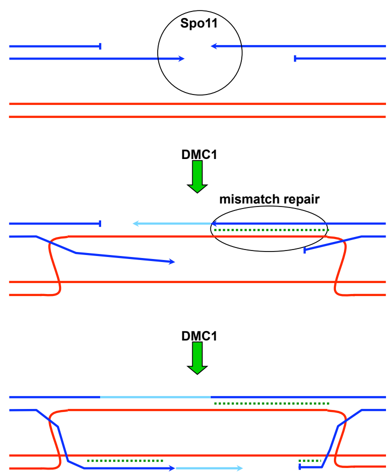


Expression of γH2AX in the testis.
Two-dimensional gel analysis of γH2AX in liver (***a***) and testis (***b***) of 5-week male mice.
Under non-irradiated conditions, γH2AX is absent from liver, but is present in testis. Phosphorylation occurs at two SQ motifs located in the carboxy-terminal portion of γH2AX, and this gives rise to two differentially migrating γH2AX species indicated by the arrows in (*b*).

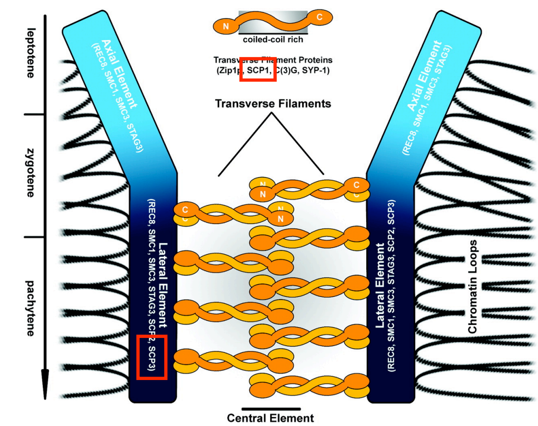
γH2AX expression is shown in squashed spermatogenic cells (*c–f*, DAPI, blue; *g–j*, XMR, red; *k–n*, γH2AX, green). Sertoli cells and spermatogonia (***c***) are negative for the spermatocyte-specific marker XMR (ref. 11) (***g***) and for γH2AX (***k***). In early prophase spermatocytes (leptotene-zygotene) (***d***), XMR is distributed uniformly through the nucleus without preferential staining of X and Y chromatin (***h***), whereas γH2AX displays a punctate appearance or has become restricted to a tadpole-shaped structure (***l***, arrow). As pachytene progresses (***e***), XMR becomes progressively concentrated in the sex body (***i***, arrows), and γH2AX is restricted to the sex body throughout pachytene (***m***). Round spermatids and maturing sperm (***f***) are devoid of both XMR (***j***) and γH2AX (***n***).

Recombinational DNA double-strand breaks in mice precede synapsis
Shantha K. Mahadevaiah *et al.Nature Genetics* **27**, 271 – 276 (2001)

Recombinational DSB Repair



SYNAPTONEMAL COMPLEX COMPONENTS



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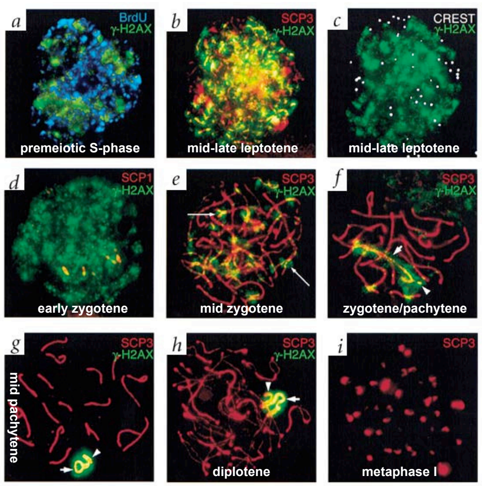
DSBs Precede Synapsis



Expression of γH2AX during XY male meiosis.(Cells imaged, SCP3 117, SCP1 48, BrdU 34; SCP3 or SCP1 (*d*), red; γH2AX, green; BrdU, pseudocolored blue; CREST marking centromeres pseudocolored white; colocalization, yellow; arrowhead, Y chromosome; short arrow, X chromosome). ***a***, BrdU-stained premeiotic S phase nucleus with a number of γH2AX domains. We found that 44% of BrdU-positive cells judged to be in premeiotic S on morphological grounds were γH2AX positive. ***b***, Mid-late leptotene nucleus. γH2AX is abundant encompassing a large proportion of the developing axial elements. Nevertheless, there are clear 'pockets' of axial elements lying outside the γH2AX domains. ***c***, The same nucleus as in (*b*) showing showing the location of the centromeres as revealed by CREST; the centromeres are clustered within the γH2AX deficient pockets. ***d***, Very early zygotene nucleus. There are only a few very short stretches of SCP1-positive SC, but there is extensive γH2AX staining. ***e***, Mid zygotene nucleus. γH2AX staining decreases and becomes restricted to the chromatin of the last axial elements to synapse (long thin arrows). ***f***, Nucleus at zygotene-pachytene transition. γH2AX has disappeared from the autosomal chromatin but is now associated with the condensing X and Y chromatin (the tadpole-shaped structure). **g**, Mid pachytene nucleus. γH2AX is present only in the sex body. ***h***, Diplotene nucleus. γH2AX remains in the sex body as it begins to migrate to the center of the nucleus. ***i***, Metaphase I nucleus. γH2AX has disappeared.

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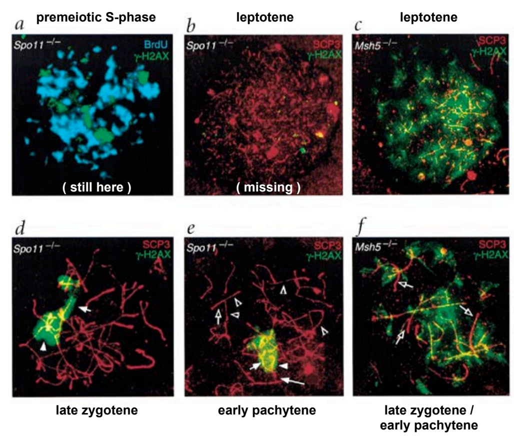
γH2AX Requires Spo11 (mostly)
MMR required for homologous synapsis



Expression of γH2AX in spermatogenic cells from *Spo11-/-* and *Msh5-/-* mice.(Cells imaged, *Spo11*-/- 40 + 56 BrdU, *Msh5*-/- 53; SCP3, red; γH2AX, green; BrdU pseudocolored blue; colocalization, yellow; arrowhead, Y chromosome; short arrow, X chromosome). ***a***, *Spo11*-/- BrdU-stained premeiotic S-phase nucleus. γH2AX staining is retained. We found that 43% of BrdU positive premeiotic S phase nuclei were γH2AX positive. ***b***, *Spo11*-/- leptotene nucleus. γH2AX is almost completely absent. ***c***, *Msh5*-/- leptotene nucleus. In contrast to the *Spo11*-/- mutant, there is abundant γH2AX. ***d***, *Spo11*-/- late zygotene nucleus with a mixture of synapsed and asynapsed axes. γH2AX is restricted to the 'tadpole-shaped' sex chromatin. ***e***, *Spo11*-/- early pachytene nucleus (stage judged from condensing sex chromatin and extent of PAR synapsis, long arrow). γH2AX is absent from the autosomal chromatin, whether synapsed (open arrows) or asynapsed (open arrowheads). The sex chromatin remains stained, except for the synapsed PAR. ***f***, *Msh5*-/- late zygotene/early pachytene nucleus. Although much γH2AX remains, it is lost in regions of synapsis (open arrows).

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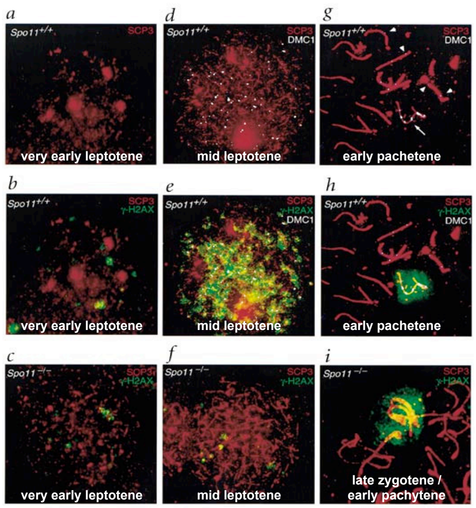
No Recombination without Spo11



The relationship between γH2AX domains, axial elements and DMC1 foci during leptotene and zygotene.(Cells imaged, *Spo11*+/+ 49, *Spo11*-/- 71; γH2AX, green; axial elements as revealed by anti-SCP3, red; DMC1 foci, pseudocolored white.) ***a***, *Spo11*+/+ very early leptotene nucleus (with the γH2AX signal removed). DMC1 foci have not yet appeared on the newly forming axial elements. ***b***, Same nucleus with the γH2AX signal, showing discrete γH2AX domains separate from the axial elements. ***c***, *Spo11*-/- very early leptotene nucleus. Once again DMC1 is absent, whereas γH2AX is present in discrete domains. ***d***, *Spo11*+/+ mid leptotene nucleus (with γH2AX signal removed). DMC1 foci are now abundant on axial elements. ***e***, Same nucleus with the γH2AX signal, which locates preferentially to the chromatin of DMC1-positive axial elements. The γH2AX negative pockets are those shown to be occupied by the clustered centromeres. **f**, *Spo11*-/- mid leptotene nucleus. DMC1 is undetectable and the γH2AX signal remains very sparse. ***g***, *Spo11*+/+ early pachytene nucleus (with γH2AX signal removed). DMC1 foci (arrowheads) have disappeared from many but not all of the newly synapsed autosomal bivalents but remain on the largely asynapsed X chromosome (arrow). ***h***, Same nucleus with the γH2AX signal, which is now restricted to the chromatin of the sex body. ***i***, *Spo11*-/- late zygotene/early pachytene sex body. A strong γH2AX signal is present over the X and Y chromatin, but no DMC1 is detectable.

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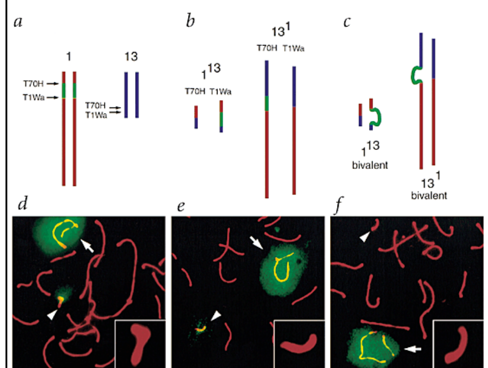
Chromosome rearrangements cause meiotic difficulties: Molecular speciation?



Karyotype (*a–c*) and expression of γH2AX (*d–f*) in T(1;13)70H/T(1;13)Wa double translocation heterozygotes. (Cells imaged, 55 SCP3, red; γH2AX, green; colocalization, yellow; 113 bivalent, arrowhead; sex body, short arrow.) The 113 bivalent from (*d–f*) is shown as an inset without γH2AX signal. ***a***, T70H and T1Wa breakpoints on chromosomes 1 and 13. ***b***, Result of the double translocation, which gives rise to two largely homologous bivalents, each with a non-homologous chromosome 1 segment (green). ***c***, The 113 and 131 heteromorphic bivalents. During meiosis, each bivalent frequently has an initially asynapsed axial loop comprising the non-homologous chromosome 1 region. ***d***, Pachytene nucleus from translocation heterozygote showing 113 bivalent with asynapsed axial loop. The chromatin of the asynapsed loop is γH2AX positive. ***e***, Pachytene nucleus from translocation heterozygote showing synapsed 113 bivalent with remnants of γH2AX. ***f***, Pachytene nucleus from translocation heterozygote showing 113 bivalent with complete non-homologous synapsis of the axial loop; γH2AX has disappeared. (Note: the heteromorphic 131 bivalent is not seen in *d–f*, because it adjusts earlier than the 113 bivalent.)

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