2
Genome Dynamics and Stability

Series Editor: Dirk-Henner Lankenau

Recombination and Meiosis

Crossing-Over and Disjunction

Volume Editors: Richard Egel, Dirk-Henner Lankenau

With 47 Figures

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Cover

The cover illustration depicts two key events of DNA repair: 1. The ribbon model shows the structure of the termini of two Rad50 coiled-coil domains, joined via two zinc hooks at a central zinc ion (sphere). The metal dependent joining of two Rad50 coiled-coils is a central step in the capture and repair of DNA double-strand breaks by the Rad50/Mre11/Nbs1 (MRN) damage sensor complex. 2. Immunolocalization of histone variant γ-H2Av in γ-irradiated nuclei of *Drosophila* germline cells. Fluorescent foci indicate one of the earliest known responses to DNA double-strand break formation and sites of DNA repair.
(provided by Karl-Peter Hopfner, Munich and Dirk-Henner Lankenau, Heidelberg)

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Preface

Volume 1 of the current series on *Genome Dynamics and Stability* identified *Genome Integrity* as the *non plus ultra requirement* for cellular life. Whether it is extracellular viral genomes, cellular prokaryotic or eukaryotic genomes, the integrity of genomes is the precondition for all life. This criterion is reflected in the underlying biochemical DNA/RNA metabolism processes, mainly represented by DNA/RNA replication/transcription and DNA repair. We now present the second book of this series. It deals with *Recombination and Meiosis: Crossing-Over and Disjunction*. It will soon be accompanied by a third book, likewise dealing with recombination and meiosis, but focusing a little more on theory–practice coupled approaches. The title of the third book will be: *Recombination and Meiosis: Models, Means and Evolution*.

When cells, during evolution, assembled into multicellular aggregates – a phenomenon we have to accept as a fact of complex life that has happened more than once – many of the most basic genome-maintenance factors were reshaped by Darwinian selectional forces. To be sure, long before the emergence of multicellular organisms, cyclic mechanisms became established to combine two haploid genomes and to reduce the diploid genome back to haploid ones. Yet, the relative abundance of haploid versus diploid stages remained highly variable. After billions of years of unicellular evolution, within a lineage stemming from a diploid protist with gametic meiosis, the origin of modern metazoans began in a (pre)cambrian diversification (i.e. explosion) to multicellular diversity where selectional forces always had a broad spectrum of molecular factors, phenomena and mechanisms to act upon. Among the molecular and cellular key processes making multicellular complexity possible were i) the potentially immortal germline from which somatic cells differentiate and ii) meiosis to precisely half the number of chromosomes established in the zygote. The differentiation of gametes into resourceful, immobile eggs and highly motile sperm cells probably developed very early in the metazoan lineage. In a certain, evolutionarily meaningful, way the animal body can be considered the germ cells' most successful means of being nourished and disseminated.

As a cytogenetic phenomenon preceding gametogenesis, where homologous chromosomes undergo programmed crossing-over and recombination, meiosis has been known since the early days of the chromosome theory of inheritance, but only more recently have the underlying molecular processes

become accessible. The present book focuses on crossing-over between and disjunction of chromosomes during the meiotic cell cycle.

The first chapter is an introductory overview written by Richard Egel, the initiator of this twin-volume edition; this synopsis covers the scope of both accompanying books. The second chapter by José Suja and Julio Rufas deals with the highly condensed cores of mitotic and meiotic chromosomes, their supramolecular structures and the involved segregation processes. Written by these leading specialists on visualizing the core structures by silver staining, it presents the current view on the relationship between the chromatid cores and the synaptonemal complex lateral elements, DNA topoisomerase IIα, and the glue between individual chromosomes, i.e. condensin and cohesin complexes, is assessed. The third chapter is written by Koichi Tanaka and Yoshinori Watanabe. It represents pioneering work in unraveling the molecular systems of chromatid cohesion. We are here confronted with key questions as to how mono-oriented sister kinetochores attach to microtubules, each to only one cellular pole, and how sister chromatids separate during meiosis I, while homologs remain paired until their segregation in meiosis II. The centrally important key proteins are presented. The fourth chapter is written by another pioneer, Scott Keeney, who discovered the DNA double-strand break (DSB) initiating Spo11 protein in yeast and the mechanism involved in how chromosomes initiate programmed recombination during meiosis by means of this archaeal-like topoisomerase. The fifth chapter by Sonam Mehrotra, Scott Hawley and Kim McKim deals with *Drosophila* as a metazoan model organism providing molecular, genetic and cytological details on how meiotic pairing and synapsis can proceed independently of programmed DSBs in DNA. Further, it elucidates the relationship of DSB formation to synapsis, how crossovers are determined and formed, and the role of chromosome structure in regulating DSB formation and repair, including specialized pairing sites. The chapter by Terry Ashley deals with recombination nodules in mammalian meiotic chromosomes and the dynamics of shifting protein compositions, while cytological structures remain nearly constant. The seventh chapter by Celia May, Tim Slingsby and Sir Alec Jeffreys exploits the human HapMap project to shed light on recombinational hot spots in human chromosomes during meiosis. The eighth chapter by Haris Kokotas, Maria Grigoriadou and Michael Petersen reviews our current understanding of human chromosomal abnormalities, as caused by meiotic nondisjuction, using Trisomy 21 as a case study.

While metazoans dominate the chapters so far – with some recourse to yeasts – plants represent another multicellular kingdom of life. In the ninth chapter Gareth Jones and Chris Franklin focus on botany's most prominent model system, i.e. *Arabidopsis thaliana*. It reviews meiotic recombination, chromosome organization and progression in this model plant, which of course, stands in for the key role of plants in agricultural production. Finally, Livia Pérez-Hidalgo, Sergio Moreno and Christina Martin-Castellanos link the meiotic program to modified aspects of mitotic cell cycle control. It reviews how mitotic regulators

adapt or are co-opted to the functional necessities of the meiotic program, paying particular attention to meiosis-specific factors whose functions are essential for meiosis. This comparative review is rooted in the pioneering cell-cycle studies on baker's yeast (*Saccharomyces cerevisiae*) and fission yeast (*Schizosaccharomyces pombe*), from where it extends to mammalian gametogenesis and other multicellular eukaryotes. A similar range of model studies has also applied to the scope of the chapter by Tanaka and Watanabe and the review of Scott Keeney.

Following the contents table of this book, the list of forthcoming chapter titles in the accompanying volume is included in advance. In fact, as some of the individual chapters had been published online first, before the editorial decision to divide the printed edition into two books was taken, the preliminary cross-references had not yet accounted for the split. We apologize for any inconvenience this may cause, but the listing of all the chapter titles in both books should hopefully direct the reader to the proper destination. We would also like to point out that the missing chapter numbers are not neglect but reflect an obligatory compromise necessitated by publishing all the manuscripts OnlineFirst immediately after they have been peer reviewed, revised, accepted and copy edited (see, http://www.springerlink.com/content/119766/).

We most cordially thank all the chapter authors for contributing to this topical edition of two accompanying books. Without their expertise and dedicated work this comprehensive treatise would not have been possible. Receiving the incoming drafts as editors, we had the great privilege of being the first to read so many up-to-date reviews on the various aspects of meiotic recombination and model studies elucidating this ever-captivating field. Also, we greatly appreciate the productive input of numerous referees, who have assisted us in striving for the highest level of expertship, comprehensiveness and readability.

We are also deeply indebted to the Springer and copy-editing staff. In particular, we would like to mention Sabine Schreck, the editor at Springer Life Sciences (Heidelberg), Ursula Gramm, the desk editor (Springer, Heidelberg), and Martin Weissgerber, the production editor (LE-TeX GBR, Leipzig).

Copenhagen, Richard Egel
Ladenburg, July, 2007 Dirk-Henner Lankenau

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Meiotic Crossing-Over and Disjunction: Overt and Hidden Layers of Description and Control

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Abstract Sexual reproduction is observed in the vast majority of eukaryotic organisms. Foremost, this includes animals, plants, and fungi. In the course of sexually propagated generations, the regularities of Mendelian genetics and the segregation of partly recombined chromosomes at meiosis are two complementary faces of one and the same coin. This chapter opens the first book of two in a series, both volumes being dedicated to the complex process of meiotic recombination. This editorial synopsis focuses on the various facets of meiosis from a descriptional perspective, before the specific chapters discuss the details of molecular mechanisms. Meiosis and mitosis are viewed as alternative schemes of eukaryotic chromosome segregation, which supposedly have coevolved from a very early start. The structure and kinetics of meiotic bivalents depend on the formation of chiasmata between non-sister chromatids and the different stability of sister-chromatid cohesion along the chromosome arms and at the centromeres. The relevance of spindle dynamics for bivalent segregation and potential nondisjunction is discussed. Telomere clustering plays an assisting role during the intermediate phase of the bouquet arrangement. At the heart of meiotic prophase, pairing and synapsis of homologous chromosomes is accompanied by genetic crossing-over and chiasma formation. The what, where, and how of DNA exchange proceed from site facilitation via partner choice and homology search to the formation and resolution of heteroduplex intermediates. The nonrandom distribution of crossovers and chiasmata is subject to interference mechanisms at various levels. Finally, the segregation of chromosomes during meiosis I and II is accomplished by an interplay of basically mitotic proteins with meiosis-specific components.

Abbreviations

DSB	double-strand break
ds/ssDNA	double-/single-stranded DNA
HR	homologous recombination
MTs	microtubules
K-fiber	MT nucleated at the kinetochore
SC	synaptonemal complex

1
Characteristics of Meiotic Segregation

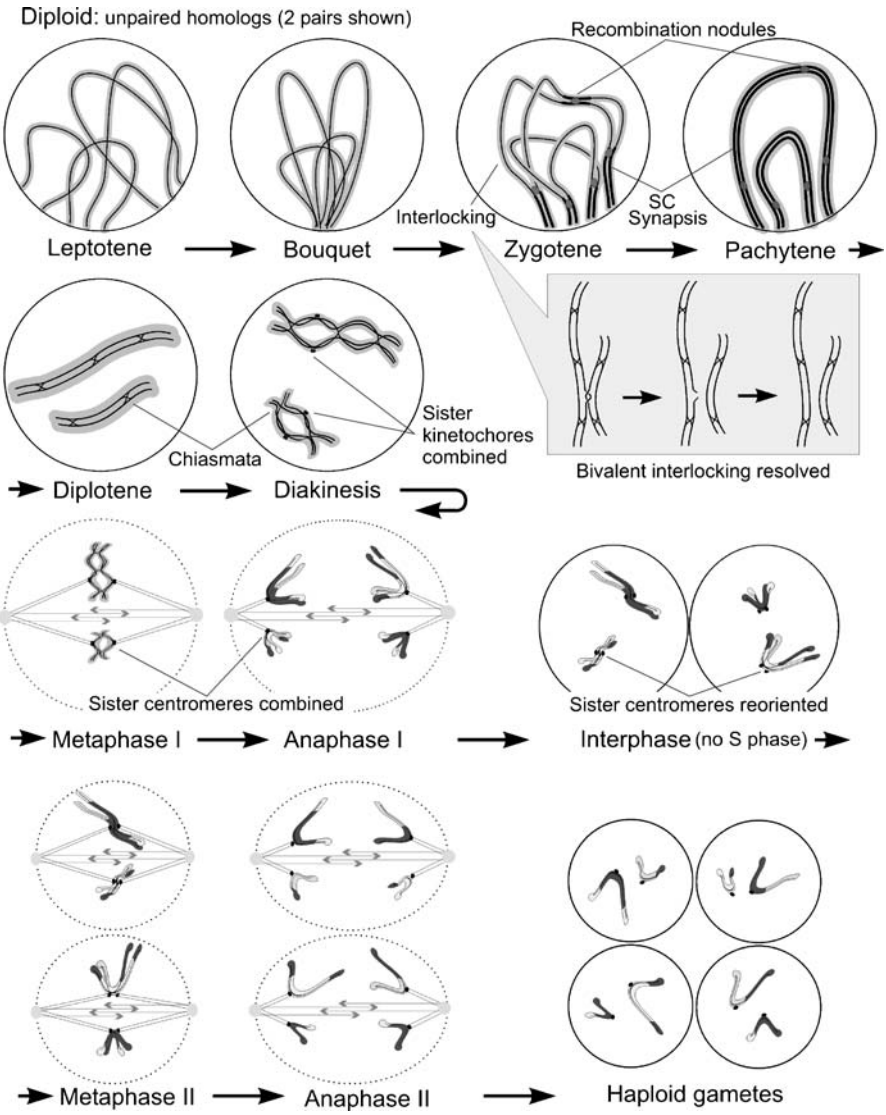
Intricate duplicity reduced

The life cycle of sexually propagating organisms alternates between two modes of nuclear division, mitosis and meiosis. While mitosis is the "working horse" of identical cell proliferation, usually repeating itself for many divisions in a row, meiosis has more exclusive rights, just once per life cycle (Sect. 2). Also, meiosis requires diploid cells, takes more time, and is more complicated at various levels; thus meiosis is more difficult to describe in straightforward and yet unambiguous terms. As in mitosis, meiosis is preceded by a round of DNA synthesis, but this single replication is followed by two rounds of chromosome segregation and nuclear division in a row – meiosis I and II. Uniquely to mainstream meiosis, a major part of the long-lasting meiotic prophase is devoted to intricate pathways of genetic recombination and chiasma formation, before the reshuffled chromosomes are segregated in two rounds. There are two main components to the meiotic redistribution of genetic material. (i) The parental chromosomes, as defined by their centromeres, are reassorted independently. (ii) The parental gene combinations on the chromosomal arms are further scrambled by crossing-over, the number position of which can vary from meiosis to meiosis. The overall result leads to four haploid postmeiotic nuclei, reducing the ploidy by half (Fig. 1).[1]

Fig. 1 Main stages of meiosis. *Leptotene*: Axial cores are visible along the chromosomes; sister chromatids are still intimately united. *Bouquet* arrangement: All the telomeres are clustered in a narrow region at the inner membrane of the nuclear envelope. *Zygotene*: Synaptonemal complexes (*SC*, marking homolog synapsis) are initiated at terminal and/or interstitial nucleation points. Recombination nodules appear, marking sites of potential chiasmata. Topological interlocking of two or more bivalents is not infrequent. *Inset*: To resolve an interlock, one of the axial cores must be broken (i.e., both sister chromatids). After the entrapped bivalent has escaped, the double-gap must be sealed, probably facilitated by SC closure. *Diplotene*: SCs disintegrate, individual chromatid cores become visible close to chiasmata. *Diakinesis*: Homologs separate, except at chiasmata; chromatid cores separate along the chromosomes, except at the centromeres. *Meta-/Anaphase I*: Fused sister kinetochores segregate to the same pole to separate the bivalents; outer chromatid arms are partly recombined. *Interphase*: There is no S phase; sister kinetochores reorient to opposite sides of each chromosome. *Meta-/Anaphase II*: Sister kinetochores segregate to opposite poles, thus producing four *haploid* gametes ▶

[1] This introductory chapter provides a synoptic view over the entire field and the topical chapters to follow, with no intention of duplicating the many references to original work cited therein. Cross-references to other chapters in this volume are cited as "this BOOK" or, if placed in the accompanying volume, as "this SERIES" (see extended "Table of Contents" preceding this chapter).

In this chapter, the description of meiotic mechanisms is focused on two main components: the transient reorganization of centromeres and the reshuffling of chromosome arms by chiasmata. In certain deviations from the mainstream regimen, one of these aspects can be observed without the other, which can make the task of an unambiguous description less difficult. The classical model organism of formal genetics, the fruit fly *Drosophila melanogaster*, follows the mainstream pattern only in female meiosis, whereas the males perform spermatogenesis without genetic



crossing-over or chiasma formation (achiasmatic disjunction).[2] Hence, the simplified version of achiasmatic meiosis is presented first.

1.1 Kinetic Activity at the Centromeres

Splitting the deal

In general, the occurrence of meiosis before the formation of germ cells serves two major objectives, the halving of chromosome number and the reshuffling of chromosomal gene contents. How is the halving by number accomplished in the achiasmatic meiosis of *Drosophila* males?

Before all the chromosomes can be disjoined in order, the pairs of homologs must physically communicate. In male meiosis this is solely accomplished by interconnections at special pairing sites[3] (McKee 1998), which appear to require transcription to be active. Two meiotic proteins have been shown to be involved in this conjunction, one being related to cohesin proteins (Thomas et al. 2005). These connections have to persist until metaphase of meiosis I. At the crucial steps of metaphase and anaphase it is important that the centromeres are organized differently in meiosis I as compared to mitosis, in that sister kinetochores are fused as a functional unit (J.A. Suja and J.S. Rufas, this BOOK). This is the same in male and female meiosis of *Drosophila*. In consequence, both sister kinetochores attach to the same spindle pole, and the kinetochores of the connected homolog attach to the other pole. At anaphase I, therefore, sister kinetochores are drawn to the same pole; both sister chromatids of each chromosome thus stay together entirely and are separated from both chromatids of the homolog.

In the short interphase between meiosis I and II, the centromeres reorganize so that sister kinetochores again are separated and face in opposite directions, as in mitosis. In consequence, they attach to spindle fibers from opposite poles, and the sister chromatids with all their genes then segregate from one another at anaphase II. The latter condition, in particular, no longer holds for chiasmatic meiosis, where the sister chromatids are broken up and scrambled by reciprocal exchange between the homologs.

[2] Another form of achiasmatic meiosis occurs in oocytes of the silkworm *Bombyx mori*, where a modified synaptonemal complex (Sect. 5) ensures the stabilization of bivalents until metaphase I. Significantly, if chiasmatic meiosis is restricted to one gender only, it is usually the "heterogametic" gender that no longer undergoes crossing-over and chiasma formation, such as in XY-bearing *Drosophila* males and WZ-bearing *Bombyx* females. This differential suppression of crossing-over has likely resulted from selection against recombinational rearrangements between the diverged sex chromosomes.

[3] Similar pairing sites may also be involved the early stages of chiasmatic meiosis of *Drosophila* females or other organisms (Sect. 5), but their influence does not usually persist until metaphase.

1.2 The Structural Relevance of Chiasmata

Sister ain't your sister, but ...

The chiasmata observed in mainstream meiosis serve both a genetic purpose (Sect. 8) and a structural role for the segregation mechanism itself. Without chiasmata, the paired up homologs (termed bivalents) would fall apart before metaphase. Each individual chromosome would then be free to attach to either spindle pole, independently of its homolog[4], approaching a 50% risk of "nondisjunction", when both coincidentally are gathered at the same pole (H. Kokotas, M. Grigoriadou and M.B. Petersen, this BOOK). The structural glue that manifests itself by bivalent stability in the presence of chiasmata can be ascribed to sister-chromatid cohesion (K. Tanaka and Y. Watanabe, this BOOK), notably in the distal parts of the chromosome arms, facing away from the centromeres (J.A. Suja and J.S. Rufas, this BOOK).

This is a formidable challenge to a fairly common mantra of meiosis, that *sister chromatids stay together in meiosis I, only to be separated equationally in meiosis II*. While this description, in fact, is fully valid for achiasmatic meiosis (Sect. 1.1), it no longer fits unconditionally for the mainstream form of chiasma-based meiosis (Fig. 1). To save the relevant part of the commonly repeated phrase, and do justice to the fundamental importance of meiotic chiasmata as well, it is necessary to observe the following qualifications.

With due consideration of the local constraints imposed by the chiasma, the said notion can still be applied to the sister kinetochores themselves and the adjacent segments of sister chromatids, up to the first chiasma on either side. For these innermost parts alone, disjunction at meiosis I will always be reductional. For the next segments, between the first and the second chiasma, sister chromatids are always segregated in meiosis I already. Yet, further out beyond the second chiasma, it will be 50 : 50 whether sister chromatids separate in meiosis I or II. Ironically, therefore, where sister-chromatid cohesion is most important for bivalent stability in metaphase I (just distal of the first chiasma from the centromere), these parts of sister chromatids will *never* stay together in anaphase I. On average, therefore, only half the genes in the genome will follow the segregational pattern laid out by the centromeres, that *sister kinetochores stay together in meiosis I*, only to be separated equationally in meiosis II; the other half will just do the opposite.

Sister chromatid cohesion is critical in providing the structural support for bivalent stability at metaphase. It balances the pulling forces exerted by spindle fibers towards the spindle poles (Sect. 9). Eventually, though, this cohesion must dissolve, thus giving way to the segregational movements at

[4] In several organisms, recombination-independent centromere association can still favor proper homolog disjunction to some extent (Davis and Smith 2003; see D.Q. Ding and Y. Hiraoka, this SERIES).

anaphase. This release is mediated by proteolytic cleavage of a connecting subunit in the cohesin complex. As a characteristic modification at meiosis, the release of sister chromatid cohesion occurs in several steps, for different parts of the chromosomes (K. Tanaka and Y. Watanabe, this BOOK). At first, at the metaphase/anaphase transition of meiosis I, it is only dissolved along the arms. This releases the topological constraints at the chiasmata where the partly exchanged chromatids had been physically interlaced. Around the centromeres, however, the cohesin complexes remain intact until they are dissolved at the metaphase/anaphase transition of meiosis II.

Other structural changes concern the topology of so-called chromatid cores, which form the connecting threads in a radial-loop/scaffold model of chromatin organization in chromosomes (J.A. Suja and J.S. Rufas, this BOOK). These scaffolding cores consist of various proteins, such as topoisomerase II and condensin[5] complexes, respectively involved in the decatenation of interlocking DNA loops and the successive contraction of chromatid arms in the preparation for division. Very characteristically, the contraction of sister chromatids appears to proceed by "relational coiling", giving opposite helical handedness to both strands. This may effectively pry the sister chromatids apart until fewer and fewer interlocks remain to be resolved by the topoisomerase. As meiotic prophase proceeds beyond the stage of homolog synapsis (Sect. 5), the chromatid cores separate first at the sites of chiasmata. At this stage it becomes evident that a seamless reconnection has been established at the light-microscopic resolution of chromatin superstructure, reflecting the molecular exchange of the corresponding DNA molecules by a genetic crossover event. This reconnection of chromatid cores at chiasma sites is likely prepared by the so-called recombination nodules, which can be visualized by electron microscopy (and/or immunostaining for specific protein components) even at the preceding synapsis stage (T. Ashley, this BOOK).

2 The Staging of Meiosis

The ultimate alternative

The genetic exchange with matching partner chromosomes, as observed in mainstream meiosis, requires matching pairs of homologs to begin with. For a primarily haploid unicellular organism, this means that two haploid cells have to merge and combine their nuclear genomes before meiosis can commence to rearrange both sets of chromosomes.

[5] Condensin proteins are structurally related to the cohesins mentioned before, but the mechanisms of their action and control are not yet fully explored.

2.1 Life-Cycle Variants

The purely haplontic life cycle likely represents an early setting in eukaryote evolution (R. Egel and D. Penny, this SERIES). It is characterized by vegetative propagation of haploid single cells, and meiosis occurs in zygotes (zygotic meiosis), the fusion nucleus being the only diploid stage in the life cycle. Among extant eukaryotes, however, this simple scheme is not observed abundantly. Three scattered examples of this category are the social amoeba *Dictyostelium discoideum*, the unicellular green alga *Chlamydomonas reinhardtii*, and the fission yeast *Schizosaccharomyces pombe*. In these, meiosis is related to the formation of dormant resting stages, zygotic cysts in the first two cases and ascospores in the third example.

In contrast, gametic meiosis prevails in the purely diplontic life cycle of metazoans, immediately before the formation of dimorphic gametes, the female eggs, and the male spermatocytes. Accordingly, these gametes are the only haploid cells occurring in either gender, and the diploid phase is reestablished upon fertilization by sperm/egg fusion. The fertilized egg, or zygote, develops into various lines of stem cells, from which the differentiated body tissue cells derive. Typically, it is only the most universal class of stem cells that ultimately can lead to meiosis anew, thus giving rise to the next generation of germ cells. What it is at the molecular level that sets the so-called germline apart from ordinary soma cells is still under active investigation (D.-H. Lankenau, this SERIES).

In addition to the purely haplontic or diplontic extremes, a varied spectrum of mixed strategies unfolds in other organisms, where meiosis and fertilization are separated by mitotic cell divisions both at the haploid and the diploid level. Even though flowering plants (e.g., *Arabidopis thaliana*, G.H. Jones and F.C.H. Franklin, this BOOK) superficially resemble the diplontic cycle of animals, the evolutionary history relates their breeding system to alternating generations of diploid "sporophytes" and haploid "gametophytes". Yet, while both these generations can comprise many somatic cell divisions in algae, mosses[6] or ferns, the haploid gametophytes of flowering plants have been reduced to inconspicuously few nuclear divisions that are well hidden within the "female" flower parts of their diploid host plants[7], where seed formation is initiated.

[6] In mosses and horn-worts, the life cycles are actually dominated by the habitus of the haploid gametophyte stage.

[7] In this nomenclature, all the visible parts of a flowering plant belong to the diploid sporophyte, which produces two kinds of haploid meiospores. The microspores or pollen grains adopt the male role in cross-fertilization, and the megaspores adopt the female role. While the megaspores of modern plants develop into fertilizable ovules directly, the microspores germinate to form a pollen tube (the male gametophyte) with two or more haploid nuclei, only one of which fuses with the ovular nucleus during fertilization.

In the third kingdom of multicellular eukaryotes, the filamentous fungi, the uncoupling of meiosis from cellular or hyphal fusion leads to yet more diverse variation, in that nuclear and cytoplasmic phases are essentially uncoupled. As to their nuclear state, most fungi actually follow a strictly haplontic cycle, where meiosis proceeds directly from karyogamy, the sexual fusion of two haploid nuclei. This is then followed by the formation of haploid spores.[8] Notably, the ultimate fusion of nuclei before meiosis is preceded by extended periods of vegetative growth where two types of haploid nuclei share a common cytoplasm.[9] The characteristic fruiting bodies of mushrooms belong to this category. At this stage, complementary gene functions can be expressed in the common cytoplasm, even though the individual nuclei remain genetically distinct and haploid. Only rather few fungi have developed regular stages of diploid growth, such as the infectious phase of plant-pathogenic smut fungi (e.g., *Ustilago maydis*) or the unicellular bakers yeast (*Saccharomyces cerevisiae*).

In addition to the multicellular members of the so-called crown group (comprising animals, fungi, and green plants) there are the numerous phyla of unicellular protists. Some of these add further variety to life-cycle strategies, and many others are not fully explored in that respect. Arguably the most interesting and complex variation is found in ciliates (such as *Tetrahymena* or *Paramecium*), which at the unicellular level operate with dimorphic nuclei of different function (see Katz 2001). Of these, transcription for protein synthesis is limited to the highly polyploid macronucleus, which typically can only last for a certain number of vegetative cell divisions. On the other hand, the diploid micronucleus is dedicated to a merely generative role. During the sexual encounter of ciliate conjugation the macronuclei are resorbed, and only the micronuclei of both partners undergo meiosis. Three of four postmeiotic nuclei are resorbed as well, and the remaining one divides at least once at the haploid level. Each conjugant cell retains one of these nuclei and exchanges the other with its partner, and the respective nuclei fuse and divide mitotically at the diploid level. Thereafter, one of the diploid nuclei is retained as the new micronucleus, and the other one regenerates the new macronucleus. In operational terms, this nuclear division of labor very much resembles the germline/soma differentiation observed in multicellular metazoans.

[8] The fungal meiospores can be formed inside a larger cell (the ascospores of ascomycetes) or be extruded from a basal cell (the basidiospores of basidiomycetes).

[9] If the sexually different nuclei associate in pairs and divide coordinately in a stereotype pattern of retrograde migration of one of the daughter nuclei, this mixed phase is called a dikaryon; otherwise, if several nuclei are contained in syncytial mycelia without pairwise coordination, this is referred to as a heterokaryon.

2.2 Cell-Cycle Reprogramming

Within the various life-cycle strategies, individual cells are programmed ahead of time as to whether their next division will follow the mitotic pattern by default, or will prepare for meiosis instead (L. Pérez-Hidalgo, S. Moreno and C. Martín-Castellanos, this BOOK). In metazoans, the entry into meiosis of germline cells is largely controlled by specialized somatic cells, such as Sertoli cells in the testicle and follicle cells in the ovary, which nurture the developing germ cells through spermatogenesis and oogenesis, respectively. In potentially immortal, unicellular organisms, however, essentially all the cells are able to switch to the sexual program of cell fusion and/or meiosis, in due response to appropriate environmental signals. Comparing different organisms in terms of cell cycle regulation in this regard, transcription factors have been recruited anew or decommissioned many times, so the contribution of regulatory components has been conserved rather poorly during evolution. Hence, I will only highlight certain superior principles for this synoptic view.

By and large, meiosis and mitosis stand out as two modular alternatives for a single cell to organize its next division. Even though numerous functional components are common to both mitosis and meiosis, others are not, and the specific ones are usually subject to multiple control systems. In brief, here are some informative examples for molecular toggle switch systems, which ensure the mutually exclusive performance of either program.

In nematodes (the roundworm *Caenorhabditis elegans*), two antagonistic signal sets direct developing germline cells towards mitosis in the beginning, or towards meiosis later on (Kimble and Crittenden 2005; Suh et al. 2006). The mitotic set comprises a Notch-type[10] membrane receptor and several RNA-binding proteins. The stimulating Notch signal originates from a single somatic cell at the tip of the developing gonad, and its strength diminishes with distance from the source. The meiosis-promoting set, on the other hand, comprises both a transcriptional and a translational repressor, a cytoplasmic poly(A) polymerase, and another RNA-binding protein. Notably, each set of regulatory factors downregulates expression of the other set. Accordingly, mitotic proliferation of germline cells prevails close to the tip cell, and meiosis is initiated in a sliding zone from the other end of the gonad. Still, among mRNAs to be controlled, the most important downstream targets that react to these signals remain to be identified.

As to free-living yeasts, every single cell is potentially capable of entering meiosis, which then is followed by ascospore formation. This occurs in response to a combination of internal and environmental signals (L. Pérez-Hidalgo, S. Moreno and C. Martín-Castellanos, this BOOK). Both fission yeast (*S. pombe*) and bakers yeast (*S. cerevisiae*) need to be heterozygous

[10] A widely conserved intercellular signalling pathway named after the *Drosophila Notch* mutant.

for mating-type genes, which ensures that only diploid cells can engage in meiosis and sporulation. Furthermore, the nutrient supply from the medium should be depleted, especially for a nitrogen source. In both these yeasts, the induction of meiosis depends on upregulation by critical (though nonhomologous) transcription factors, Ste11 in *S. pombe* and Ime1 in *S. cerevisiae*. Also, in *S. pombe*, a protein kinase (Pat1) that normally inactivates all meiotic activities in the vegetative state has to be specifically inactivated first (reviewed by Yamamoto 2004). This extra safeguard has not been observed in bakers yeast; it may be related to the predominantly haploid mode of fission yeast cells, where the inadvertent induction of meiosis would be especially precarious.[11]

Transcriptional regulation has long been considered key to understanding how the cell division machinery is switched from the ordinary mitotic mode to the meiotic alternative. Indeed, large sets of genes are preferentially expressed during meiosis, as shown by genome-wide analyses in both *S. cerevisiae* and *S. pombe* (Chu et al. 1998; Mata et al. 2002). Yet, the either/or of this bifurcation is also corroborated at other levels of control, such as differential mRNA stability (Daga et al. 2003), alternative splicing of meiotic transcripts (Juneau et al. 2007) or meiosis-specific translational control (Reynolds et al. 2007).

Studies in both model yeasts suggest that the decision to initiate meiosis has to be taken before "premeiotic" S phase. This makes DNA synthesis an integral part of the meiotic program of molecular events. What then is special about this crucial round of replication? From studies on meiosis in lily anthers it was deduced that replication of some DNA ($\gg 1\%$) was delayed from general S phase to zygotene (Hotta et al. 1985). This special DNA could then have played a role in homolog pairing and synapsis. Yet, similar findings have not since been extended by others to other organisms; so the generality of this assumption remains unproven. On the other hand, premeiotic DNA synthesis need not be different as such, if only the critical processes happened to be associated with S phase. This could be the loading of ancillary protein complexes, such as meiosis-specific cohesins. As to mitotic cohesins, it has indeed been shown that sister-chromatid cohesion is established at replication forks, after the necessary loading of cohesin complexes has occurred before S phase (Uhlmann and Nasmyth 1998; Lengronne 2006).

Later on, the direct succession of meiosis I and II (without an intervening round of DNA replication) requires a delicate balancing of cyclin-dependent protein kinases and other regulatory factors (L. Pérez-Hidalgo, S. Moreno and C. Martín-Castellanos, this BOOK). Moreover, the special features of meiotic prophase concerning homolog pairing, synapsis, and recombination are discussed in the following sections.

[11] Starting meiosis from the haploid state, of course, has detrimental consequences and is avoided by special safeguarding controls; conditionally lethal *pat1*^{ts} mutants can be obtained in *S. pombe*, which initiate meiosis at the nonpermissive temperature.

3
The Essence of Meiotic Recombination and Marker Exchange

Shuffling the deck

Next to reducing by half the number of chromosomes from the diploid level, the hallmark of meiosis is the exchange of genetic markers, inherited from slightly different parents. This can be due to the random assortment of nonhomologous chromosomes in meiosis I, as well as the breakage and rejoining of homologs in meiotic prophase.[12] Among the various types of genetic recombination (Box 1; J.E. Haber, this SERIES), reciprocal exchange events between homologs are especially important, since only these produce chiasmata. More detailed analyses have suggested that essentially every meiotic crossover event is associated with the formation of some heteroduplex DNA at the actual site of molecular recombination (Borts and Haber 1987). This can result in a limited segment of nonreciprocal recombination (gene conversion and/or postmeiotic segregation), together with the reciprocal exchange of all the other markers that lie outside and are not involved in heteroduplex formation. In addition, there are other events of local heteroduplex formation that do not lead to chiasmata. Such events can still be observed as a limited stretch of gene conversion, with no reciprocal exchange of outside markers.

Box 1 Glossary: Basic terms relating to genetic recombination

Recombinants	Progenies in which markers from different parents are recombined.
Assortment of chromosomes	Independent segregation of different parental chromosomes in meiosis I. Genetic recombinants can arise without the molecular recombination of DNA.
Crossing-over	Reciprocal exchange of linked genetic markers. Both types of recombinants can be recovered from the same meiosis (usually by tetrad analysis).
Gene conversion	Nonreciprocal exchange of linked genetic markers, most commonly observed as 3 : 1 segregation of two alleles in tetrad analysis.
Post-meiotic segregation	Segregation of genetic markers in the first mitosis after meiosis II, most commonly observed as 5 : 3 segregation of two alleles in extended tetrad analysis. This is attributed to the formation of heteroduplex DNA as a recombinational intermediate.

[12] Thus, inasmuch as nonallelic genetic markers are carried on different chromosomes, recombinant progeny can also result from achiasmatic meiosis. For markers on the same chromosome, however, recombinants can only arise from crossing-over and chiasmata.

Box 1 (continued)

Aberrant 4 : 4 segregation	Presence of heteroduplex DNA in two chromatids of a meiotic tetrad, as inferred from post-meiotic segregation of the same allelic markers twice, in parallel mitotic divisions after meiosis II.
Chiasma	The cytologically visible result of a crossover event.
Homolog pairing	Approximate alignment of homologous chromosomes
Synapsis	Tight juxtaposition of homologs, mediated by synaptonemal complex (SC) structures
Recombination nodule	Electron-dense structure correlating with recombination events, usually associated with SCs
Bivalent	Pair of homologous chromosomes, connected by SC or chiasmata
Univalent	Single chromosome lacking chiasmata or a pairing partner

In most organisms, meiotic crossing-over can occur along most of the chromosomes, with the exception of certain cold-spot regions, such as pericentromeric heterochromatin and the rDNA repeats of the nucleolar organizer. Upon closer inspection, the actual exchange point distribution throughout the euchromatic regions is not entirely uniform, but is often marked by distinctive peaks of recombinational hotspots (C. May, T. Slingsby and A.J. Jeffreys, this BOOK). Some of these hotspots may be due to preferential sites of DNA breakage, but preferential resolution of other recombinational intermediates may also be involved.

4 The Enigma of Partner Choice

Welcome, Parvenu!

Essentially all the major players in the molecular pathways to meiotic crossing-over are either identical with enzymes involved in recombinational repair in mitotic cells, or evolutionarily related to such activities (W.D. Heyer, this SERIES). Yet, the "damage" that needs repair, and thus triggers meiotic recombination, is by no means accidental. In contrast to endogenous or environmental DNA damage, which may hit any cell at any time, the double-strand breaks (DSBs) that appear to be required for meiotic crossing-over are catalyzed by a special enzyme that has no other function in the life cycle of the organism. First discovered in yeast, the Spo11 family of proteins is homologous to topoisomerase VI from Archaea. Differently from ordinary endonucleases, a Spo11 dimer does not leave its substrate after the reaction,

but remains covalently attached to the 5′ ends at either side of the break (S. Keeney, this BOOK). Hence, the cut DNA is not subject to unrestrained extension of the damage, but is directly processed further by one of several repair pathways in a carefully controlled fashion.

When a diploid mitotic cell, at G_2 of the cell cycle[13], suffers DNA breakage, it has several choices for staging a templated repair process: it can either choose the corresponding homolog (two chromatids available) or the fully identical sister chromatid. As the sister chromatids in G_2 are still intimately connected by cohesion, whereas the homologous chromosomes are usually further apart, the templated repair in G_2 is strongly biased towards the sister chromatid. If a meiotic cell would naively apply the same mechanism by default, repairing the Spo11-induced DSBs off the sister as a template, this would have no effect genetically at all; so this in not a common option. Crossover-type exchange events require productive interaction with the homolog instead. Somehow, the potential recombination with the sister chromatid has to be actively suppressed, in spite of its close proximity, but how this happens is still under active investigation.

Some circumstantial evidence exists in *S. pombe* that the Spo11 equivalent is loaded onto DNA together with the establishment of sister-chromatid cohesion (G. Cromie and G.R. Smith, this SERIES). In budding yeast, this cohesion is established during S phase (Uhlmann and Nasmyth 1998; Lengronne et al. 2006). These cues may be the most relevant for grasping the molecular basis for partner choice bias in meiotic crossing-over. Also in budding yeast, the screening for partner choice mutants has pointed at several relevant candidates (Thompson and Stahl 1999). Among other functions, a meiosis-specific checkpoint kinase (Mek1) plays a critical role in these controls (Perez-Hidalgo et al. 2003; Niu et al. 2007). Based on the close juxtaposition between meiotic sister chromatids, an integrated model has been proposed assuming the coordinated assembly of a regional "barrier to sister chromatid repair" (BSCR) wherever a functional Spo11 complex has been loaded on to (and/or activated on) the other chromatid (Niu et al. 2005).

5
Searching for Homology

Finding the needle in a haystack

Crossing-over during meiosis is directed at interacting chromosome pairs of homologs; it is "homologous recombination" (HR) in a nutshell. Yet, to engage in HR productively at any given site, sufficient "homology" at the

[13] Relative to DNA replication (synthesis) at S phase, the interphase between mitotic divisions is described by two gaps, G_1 before replication and G_2 after.

DNA level must first be ascertained beyond a predetermined threshold, discriminating against randomly occurring shorter stretches of DNA sequence similarity within nonhomologous surroundings. Ideally, DNA homology is defined as residual sequence identity by descent, rather than the coincidental similarity arising from stochastic variation. The enzymes performing HR, however, can only go one way or the other by directly comparing two sequences at a time, with rather few independent cues as to their likelihood of sharing a common ancestry. How can such enzymes quickly and reproducibly fulfill this role?

The archetypal enzyme for assessing the degree of homology between a 3′ end of ssDNA and potential double-stranded target sequences is the RecA protein of *Escherichia coli* (C. Prévost, this SERIES). This protein is involved in DSB repair[14]; RecA orthologs exist in Archaea (RadA) and in eukaryotes (Rad51). In general, eukaryotes carry a meiosis-specific paralog (Dmc1) and may have additional Rad51 paralogs as well. These important members of a DNA-dependent ATPase family have in common that they can assemble as helical filaments on ssDNA (∼1 kb or even longer), which in turn can intertwine with dsDNA of any sequence.[15] Due to the rigid scaffolding provided by the surrounding protein filament, the target duplex DNA is stretched and partially unwound, which greatly reduces the base pair stacking forces. It also allows base pairing to switch coordinately between the strands – AT pairs first (reversibly) and GC pairs later on, when a high number of matching AT pairs indicates a sufficient degree of sequence homology along the so-called presynaptic filament (Folta-Stogniew et al. 2004). Most current models assume that the exchange of base pairing during the partner switch occurs by a sliding movement of individual bases, within the plane of their aromatic rings (C. Prévost, this SERIES). An alternative model preserves the remarkable symmetry of a quasi-quadruplex configuration[16], if base exchange occurs by flipping 180° about the stationary glycosidic bonds (Egel 2007).

A major problem, in fact, is one of great numbers. For every matching target of a long identical sequence there exist ever so many others that do not fit, and the prospective RecA filament on ssDNA cannot judge beforehand whether or not a random encounter with a potential duplex target happens to be a proper match. To find out, the searching filament actually has to fully intertwine in register with the duplex sequence and start the base pair exchange reaction over an extended length. Every futile encounter with a heterologous sequence has to be reversed completely before the next attempt can be

[14] Double-strand breaks arising from environmental damage or the collapse of stalling replication forks.

[15] In general, just three intertwining strands of DNA are accommodated inside the helical RecA filament. There is room, however, for a fourth strand to participate in the exchange reaction, without distorting the protein filament (Mazin and Kowalczykowski 1999).

[16] In cross-section, the three participating strands occupy three corners of a square.

initiated at a new site. It should be helpful, of course, if the filament could somehow "remember" and avoid the unproductive encounters tried before, but RecA alone is not capable of unidirectional scanning along a duplex target for a suitable start (Adzuma 1998). Additional proteins may serve as processivity factors to raise the efficiency of RecA-type filaments in this regard.

In eukaryotes, a series of other proteins associate with Rad51 and/or Dmc1 filaments, and all of them are required for full recombinase activity. Thus, one of them ("RadX") might act as a processivity factor. In brief, the RadX-modified Rad51 filament could work as follows: Instead of wasting valuable time with probing heterologous sites at any length, the 3′ end could sweep along a narrow sliding window until it finds a perfect match. The sudden drag arising from many flipped base pairs could then allow the sliding window to be widened for probing further down the line. In the rare case of having found the perfectly homologous target, this should launch an avalanche of instantaneous fit, comprising essentially all the base pairs in a row. When such a perfect match has been accomplished, the RadX–Rad51 filament is disassembled and the stretch of heteroduplex DNA is passed on to other protein complexes for further processing.

A candidate of particular interest among the recombinase-associated factors is Rad52. Among the corresponding mutants, lack of Rad52 has the strongest effect, working relatively early in presynaptic filament formation (New et al. 1998), and the entire series is termed the *RAD52* epistasis group of genes. Also, both yeast and human RAD52 proteins form heptameric ring structures, which bind preferentially to the ends of ssDNA (Shinohara et al. 1998; Parsons et al. 2000); this should be the most suitable site for a processivity factor.

6
Homolog Pairing and Synapsis

Keep in touch!

Trying to understand the initial strand exchange reaction between homologous DNA molecules may appear intricate enough; the choreography and orchestration of meiotic crossovers at the levels of chromatin and entire chromosomes is yet a different matter. In general, crossovers are not placed randomly along the chromosomes, but the molecular mechanisms behind the biased choice are still not fully understood. Both positive and negative factors influence the bias.

As each bivalent of homologs should at least have one chiasma, initial factors tend to raise the chances of getting one. For instance, physical tethers can connect the pairs of homologs after an incidental first encounter, which thereafter reduces the risk of drifting apart, thus increasing the chances for

further productive encounters elsewhere on the same bivalent. Several successive steps can be distinguished, such as initial recognition, presynaptic alignment, and synapsis (S. Mehrotra, R.S. Hawley and K.S. McKim, this BOOK; N. Hunter, this SERIES; Tesse et al. 2003; Lui et al. 2006). Only the initial recognition occurs independently of Spo11 activity (creating DSBs along the chromosomal DNA). The approximate alignment (also referred to as the pairing stage) and full synapsis by the widely conserved synaptonemal complex (SC) often require DSBs and DNA-dependent interactions. The initiation of pairing and/or synapsis on individual chromosomes can vary greatly between different organisms and even between genders of the same species. In human males, for example, initiation of pairing and synapsis invariably starts close to the telomeres (Brown et al. 2005). This correlates well with male-specific differences in the genetic map, as well as the distribution of chiasmata. Both crossing-over and chiasmata are preferentially observed close to the telomeres in male meiosis, in contrast with a more interstitial distribution during oogenesis in females (C. May, T. Slingsby and A.J. Jeffreys, this BOOK). This male-specific favoring of subterminal chiasmata may be related to the pseudoautosomal pairing regions (only 2.7 and 0.33 Mb) at either end of the otherwise nonhomologous X and Y chromosomes. The obligate chiasma observed in the major one of these makes this the "hottest" hotspot region in the entire human genome.[17] Although the independent initiation of synapsis at multiple interstitial sites has not yet been demonstrated in human oogenesis, it has been shown for numerous species with more readily accessible meiotic material (von Wettstein et al. 1984).

The occurrence of recombination-independent pairing sites is prominent in the achiasmatic meiosis of *Drosophila* males, where these contacts alone can stabilize the bivalents until metaphase I (Sect. 1.1). Preferential pairing sites of lesser stringency are also known for *Drosphila* females (S. Mehrotra, R.S. Hawley and K.S. McKim, this BOOK). In a yeast, too, pericentromeric heterochromatin association can act as a meiotic pairing site (Davis and Smith 2003). Also in fission yeast, a particularly striking example is at the *sme2* locus, which encodes a nontranscribed RNA required for the progression through meiosis. Notably, the RNA-binding inducer protein of meiosis, Mei2, aggregates specifically as a dot structure at the *sme2* locus (Shimada et al. 2003). The functional *sme2* locus has since been shown to act as a strong recombination-independent pairing site (D.Q. Ding and Y. Hiraoka, personal communication). This demonstrates that a particular RNA can organize a nucleation center for homolog pairing at the site of its transcription. At a different level, the association of meiotic telomeres to the nuclear envelope and their preferential clustering in the widely conserved bouquet arrangement (Sect. 6) can likewise increase the chances of homologous loci approaching one another in meiotic prophase.

[17] The minor region of 0.33 Mb only contributes with one chiasma per 25 meioses.

The most conspicuous identifier of meiosis at the ultrastructural level is homolog synapsis as detected by the presence of SCs.[18] With rather few exceptions, the uniform SC structure is observed in every branch of eukaryotes, and some of its components belong to the conserved core set of meiotic proteins (Villeneuve and Hillers 2001). It is assembled processively from few starting points by connecting the axial cores of homologous chromosomes with fibrous proteins across the central space. In the lateral elements of the SC, the core structures of sister chromatids are still intimately united (J.A. Suja and J.S. Rufas, this BOOK), and the individual chromatid cores are only separated after SC structures have been disassembled at the diplotene stage.

As to the actual role of the SC in mainstream meiosis, the predominant view has long been that its main function should be crucial in facilitating crossing-over by keeping the homologs in register. The cause–effect relationship, however, no longer appears to be so simple, and not all organisms behave the same in this regard. While *Drosophila* indeed requires the SC to initiate the meiosis-specific DSBs that precede meiotic crossing-over (S. Mehrotra, R.S. Hawley and K.S. McKim, this BOOK), this dependency appears to be reversed in yeast (Henderson and Keeney 2004; S. Keeney, this BOOK). One way or the other, the transformation of selected DSBs into chiasmata, including the substantial restructuring of chromatid cores with these events, appears to occur in close association with the synaptonemal complex. On the other hand, the zipper-like assembly of SCs can be quite independent of local DNA homology, which is especially evident in structural heterozygotes for chromosomal rearrangements, where normal-looking SC structures can be observed between heterologous segments (von Wettstein et al. 1984).

SC formation and recombination can also be uncoupled in other exceptional cases. In the achiasmatic meiosis of *Bombyx mori* females, SC structures are modified and stabilized until metaphase/anaphase I, when compacted chunks of central-component material are liberated as so-called elimination chromatin (Rasmussen 1977a). Conversely, in the asynaptic meiosis of fission yeast, central SC components do not form at all, in spite of high levels of crossovers per chromosome in this organism (G. Cromie and G.R. Smith, this SERIES).

If it is not crossing-over per se, could there be other important SC functions to warrant the widespread evolutionary conservation of this meiotic structure? There is, in fact, a substantial risk of physical interlocking between two or more nonhomologous bivalents. This hazard occurs if synapsis is initiated at multiple sites in the same bivalent and another chromosome arm is trapped in the middle, in turn forming an entrapped bivalent with a fourth

[18] The classical stages from light microscopy can be redefined with respect to SC formation: Leptotene, axial cores present, no SC; Zygotene, partial presence of SC, with separated axial cores in between; Pachytene, full synapsis with contiguous SCs in all the bivalents; Diplotene, disassembly of SCs, separation of lateral elements, as followed by separation of chromatid cores.