3
Genome Dynamics and Stability

Series Editor: Dirk-Henner Lankenau

Recombination and Meiosis

Models, Means, and Evolution

Volume Editors: Richard Egel, Dirk-Henner Lankenau

With 57 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

This book concludes our tandem edition on *Recombination and Meiosis*. Subtitled *Models, Means and Evolution*, it follows its first-born twin with emphasis on *Crossing-Over and Disjunction*. In the commissioning of chapter topics we have tried to cover numerous aspects of the meiotic system from many different angles.

Both these books are embedded as volumes 2 and 3 in a topical Series devoted to *Genome Dynamics and Stability*, where DNA transmission and maintenance functions are discussed from experimental and theoretical perspectives. The earlier vol. 1 dealt with *Facets and Perspectives of Genome Integrity*, focusing on DNA damage repair mechanisms, and an upcoming vol. 4 is on transposable elements. These books on meiotic processes, together with other volumes in this Series on genome management in mitotic cells, provide a grass-roots level starting platform—initiating a prospective trajectory superimposable upon the exploding field of molecular cell physiology, or *systems biology* (see below).

The preceding volume preferentially dealt with meiotic processes in multicellular organisms, such as plants and animals including man. Also, basic accomplishments from work on yeasts was presented in a comparative perspective—concerning the decisive roles of Spo11-induced breaks for crossing-over, of sister chromatid cohesion in chromosome disjunction, and cell cycle modulation in the global control of the meiotic program. The present book puts additional focus on yeasts as unicellular model organisms, where progress in revealing the mechanisms of meiotic recombination has taken place most rapidly and systematically. Also, a central aspect of genetic recombination in *E. coli* is included for its outstanding merits as a universal model. Furthermore, three facets of evolutionary relevance are also discussed.

As for the models and means of meiotic recombination, two prominent and comprehensive chapters call for particular attention. Inasmuch as theoretical interpretations of empirical data about the exchange of genetical markers in successive generations has long preceded their biochemical elucidation, James E. Haber gives expert guidance on a veritable tour de force, presenting the *Evolution of Recombination Models* from purely genetic crosses into the molecular era. He follows the historical record from simplistic breaking/joining schemes to break-induced replication, from suspected single-strand breaks to partner

choice by single-strand annealing, and from the generation of double-strand breaks (DSBs) to their repair by the establishment and resolution of single or double Holliday junctions, and finally to DSB repair in the absence of crossing over accomplished through synthesis-dependent strand annealing that does not involve Holliday junctions. This scenic ride is aptly complemented from the enzymatic perspective, as displayed by Kirk T. Ehmsen and Wolf-Dietrich Heyer on the *Biochemistry of Meiotic Recombination: Formation, Processing, and Resolution of Recombination Intermediates*. These authors highlight the biochemistry of meiotic recombination, as more and more meiosis-specific enzymes have been added to the basic toolbox, which likewise is at work in mitotic cells (cf. GDS vol. 1, this SERIES). Overlapping with functions in replication and DSB repair these enzymes[1] comprise topoisomerase, nuclease, recombinase, polymerase, and helicase activities, as well as single-strand stabilizing protein, a protective end-tethering complex and a range of modulating co-factors.

The single most remarkable feature about the initiation of meiotic recombination is the deliberate and catalyzed introduction of numerous DSBs in the chromosomal DNA. Notably, the enzyme responsible for this pivotal and conserved activity is derived from a former topoisomerase (Spo11; Keeney, this SERIES), which as such had a cell-intrinsic function essential for the untangling of replication intermediates in every cell cycle. The total number of cuts is even larger than the number of effective crossovers later on[2]. The important question of how the sites to be cut are chosen in a given cell—among myriads of potentially equivalent sites that are ignored—is still one of the most vigorously pursued aspects of ongoing research. Foremost, the susceptible substrate for meiotic DSBs is not naked DNA, but DNA embedded in chromatin, as highlighted by Michael Lichten, in his chapter on *Meiotic Chromatin—the Substrate for Recombination Initiation*. The two yeasts compared for this trait show pronounced differences in the distribution of hotspot sites for DSB formation. In *Saccharomyces cerevisiae*, a fairly promiscuous DSB machinery can be assembled at about every stretch of accessible chromatin that has been opened up for other purposes, especially at activated promoter regions. Michael Lichten coins the term "opportunistic DSBs" for these phenomena, foremost in *S. cerevisiae*—differentiating meiotic DSBs from both lower

[1] In order of appearance in the text, these actors are known to specialists by acronyms such as Spo11, Top2; Sae2/Com1, Exo1; Rad51, Dmc1; Srs2; RPA; MRX/N; Rad52, Rad54, Mnd1-Hop2, Mei5-Sae3, etc.

[2] The surplus not leading to crossing-over is eventually repaired from the sister chromatid. Intrinsically, the high value of meiotic recombination can only be compared to recombination accompanying bursts of natural transposon activation characteristic of hybrid dysgenesis syndromes (cf. Gloor and Lankenau 1998). Transposon-encoded transposases/integrases can trigger transposon excision and integration by drastically increasing DSBs and recombination rates between chromosomes—a topic highlighted in the forthcoming book of this Series. Increases in recombination can also result from irradiation-induced DSBs and other genotoxic stress (cf. GDS vol. 1, this SERIES), or during gene targeting experiments, where the free ends that trigger target DNA invasion are brought in from outside the cell.

and higher degrees of sequence specificity: on one hand ionizing radiation-induced DSBs, which occur with little sequence preference and without regard for chromatin structure, and on the other hand from the site-specific cuts of restriction-type endonucleases—or other nucleic acid transactions, such as transcription promotion, where both chromatin structure and the recognition of DNA sequence elements contribute to specificity. Such opportunistic usage of promoter-modulated open chromatin can only in part explain the DSB pattern observed in the fission yeast *Schizosaccharomyces pombe*[3], where other determinants may play a significant, hotspot-specific role. Also to be determined by meiosis-specific chromatin organization, the assembly of and/or cleavage by the DSB machinery should not be all too promiscuous on a particular issue, in that at most one of two sister chromatids can become susceptible at any given site, whereas the other sister strand needs to be protected around the equivalent site. The molecular basis for this significant restriction still remains to be determined.

After the meiosis-specific, Spo11-induced DSBs have been processed to protruding 3′ ends, these single strands have to interact with the corresponding sequence on the homologous chromosome, in order to repair and seal the break by homologous recombination. In eukaryotes the crucial strand exchange reaction is catalyzed by RecA-like recombinases of the ubiquitous Rad51 family and/or the meiosis-specific Dmc1 protein. As modeled by the most widely studied RecA recombinase of *E. coli*, Chantal Prévost, in her chapter on *Searching for Homology by Filaments of RecA-Like Proteins*, discerns their basic functions in the genome-wide search for complementary DNA strands so as to facilitate the initial strand exchange reaction in highly coordinated, helical DNA–protein filaments, which likewise are formed by the eukaryotic RecA homologs.

Corresponding studies to the leading work on meiosis in *S. cerevisiae* have also been pursued in *S. pombe*, showing striking differences in detail at various levels. The most interesting aspects of this work are pointed out in two chapters specifically devoted to the fission yeast. For one thing, *S. pombe* belongs to the rather few organisms that have lost the ability to form synaptonemal complexes in meiotic prophase, which usually stands out as the most characteristic structural basis of bivalent synapsis. Instead, another conserved feature of canonical meiosis, the clustering of telomeres in the so-called bouquet arrangement, is vastly exaggerated in a series of nuclear movements, which in *S. pombe* facilitates a dynamical alignment of homologous chromosomes from nuclear fusion throughout the entire prophase of meiosis (D.Q. Ding and Y. Hiraoka, this BOOK). Furthermore, the crossover mechanism itself is peculiar as well. Whilst many organisms including *S. cerevisiae* actually employ two partly overlapping crossover pathways, one of these pathways is entirely missing in *S. pombe*. Characteristically, the main recombinational intermediate in *S. pombe* consists of *single* Holliday junctions (G. Cromie and G.R. Smith, this

[3]The fission yeast *S. pombe* and baker's yeast *S. cerevisiae* are only rather distantly related.

BOOK), whilst earlier results on *S. cerevisiae* had suggested *double* Holliday junctions as the canonical model.

The species-oriented chapter by Gareth Cromie and Gerald R. Smith, on *Meiotic Recombination in S. pombe: A Paradigm for Genetic and Molecular Analysis*, was published Online First in June 2007. At that relatively early date, most of their extensive data on DSB hotspot distribution in *S. pombe* were mentioned in brief as unpublished results. These significant data are now more fully discussed, as mentioned above, in Michael Lichten's comparative chapter—with due reference to their recent publication in the mean time (Cromie et al. 2007). Unfortunate as such asynchrony appears to be, this is a price to pay for the advantages of Online First publication for the individual chapters as they are being completed—with a spread of Online First dates up to a year per book in such a series.

Three evolutionary topics relating to meiosis have been selected to conclude this book: the putative origin of the meiotic system, the confinement of meiosis to the germline in animals, and the abandonment of meiosis in relatively few eukaryotic lineages, some of which are remarkably persistent on the evolutionary time scale—capable of lasting for millions of years. At the dawn of genetics, crossing-over and meiosis had been considered very much the same, but the early view of apparent congruence between the two phenomena has long since been abandoned. Instead, genetic recombination as such has proved to have much earlier and more fundamental roles than the complex and highly integrated pattern of mainstream meiosis, of which crossing-over has become the most characteristic ingredient. In short, homologous DNA recombination has directly co-evolved with faithful replication (see R. Egel and D. Penny, this BOOK), clearing physical damage and/or broken replication forks as they arise (C. Rudolph, K.A. Schürer, and W. Kramer, GDS vol. 1, this SERIES)—potentially in each cell cycle of prokaryotes and eukaryotes alike. Of more sporadic occurrence, on the other hand, *meiosis* only happens once per *generation*, or *life cycle*—what ever meaning may be attached to these derived terms for unicellular organisms (see below). N.B., bacteria and archaea are proficient in recombinational repair of DSB damage to their DNA, but meiosis is missing altogether.

In multicellular organisms, the meanings of *generation* and *life cycle* are evident, and the complex inter-relationship of germline development and maintaining sexuality in animals and plants was already recognized by Charles Darwin and August Weissmann by the end of the 19th century. In his chapter on *The Legacy of the Germ Line—Maintaining Sex and Life in Metazoans: Cognitive Roots of the Concept of Hierarchical Selection*, Dirk-Henner Lankenau follows the germline concept to its historical roots, and he addresses the multiple levels of selective evolution related to this concept. Also, he fathoms Weismann's prescient usage of *germ plasm* in its original meaning that nowadays has been replaced by *genes* and *genomes*—and he sketches a tie to modern frontiers, discussing the so-called *nuage* as a germline-specific *germ plasm* or-

ganelle of multiple RNA processing, where a suspended term is thus revived in new guises.

A hallmark of meiosis is the production of recombinant offspring, efficiently scrambling the parental genotypes. The overwhelming majority of taxonomic groups throughout eukaryotes show proficiency of meiosis, at least to begin with. Higher plants and animals would probably never have originated without the evolutionary thrust empowered by meiosis. Yet, sexual propagation including meiosis has been lost repeatedly in evolution, although major evolutionary innovations have never sprung from such secondarily asexual lineages. Hence, asexual lineages of relatively ancient origins can serve as virtual mirrors to reflect the evolutionary importance of meiosis in the remaining majority of animals and plants, as thoroughly discussed by Isa Schön, Dunja K. Lamatsch, and Koen Martens in their chapter on *Lessons to Learn from Ancient Asexuals*. To single out a particular highlight, the purging of deleterious mutations by ameiotic recombination appears to be remarkably effective—readily compensating for the low mutation rates observed.

As for the inferred origin of the meiotic system, this does not only far predate the emergence of multicellular animals, fungi and plants—it even dates back before the last common ancestor of all the eukaryotic phyla known today (LECA). As canonical meiosis, therefore, is a common heritage to all eukaryotes, there are no comparative cues among different lineages living today from which by parsimony to deduce a likely order of step-wise additions to the basic toolbox of meiotic mechanisms. On the other hand, the meiotic system is so complex in its widely conserved pattern, that its instantaneous invention from scratch appears unlikely. Against this rather uninformative backdrop, Richard Egel and David Penny, in their chapter *On the Origin of Meiosis in Eukaryotic Evolution*, propose a possible series of incremental steps towards meiosis, each of which could have added some selective advantage on its own. This series may well have started before the mitotic division system had been perfected to its present fidelity, e.g. when telomere-directed chromosome movements may have preceded the establishment of centromeres. Hence their hypothesis is subtitled *Coevolution of Meiosis and Mitosis from Feeble Beginnings*. A likely driving force to establish a proto-meiotic system—alternating with proto-mitotic nuclear division—is seen in maintaining a periodically needed dormancy program, so as to protect it against the accumulation of dormancy-deficient mutations at the higher error load presumed in early evolution. This is in line with the common correlation between meiosis and the formation of dormant spores or cysts in extant microbial eukaryotes. In a certain sense, therefore, a single *generation* in the *life cycle* of unicellular eukaryotes would last from one stage of encystment or sporulation to the next.

With the commissioning and presentation of the various chapter topics on the genomic aspects of the meiotic system we hope to have served a salient need for integrating basic knowledge gained from studying diverse genetic

model organisms. Research on meiotic exchange and segregation mechanisms may appear more esoteric than the vast resources spent on understanding metabolism and growth in mitotic cells. While emphasis on the latter area is motivated by the numerical predominance of mitotic divisions, as well as the direct connection of mitotic cell divisions to the immense problems of cancerous growth in human disease, meiosis in its paucity is more secluded and its medical aspects are limited to less pressing problems, such as impaired fertility or Down-like syndromes (H. Kokotas, M. Grigoriadou, and M.B. Petersen, this SERIES). Also, a certain twist of hierarchy is undeniable: whilst endless perpetuation of mitotic divisions can be viable as an evolutionarily stable strategy, a contiguous series of several meioses is certainly not. In this sense meiosis will always be the subordinate companion of mitosis. At the conceptual level, however, the complexity of molecular mechanisms applying to meiosis far exceeds that of its mitotic counterpart. And for the continuity of generations in most eukaryotic forms of life, both meiosis and mitosis are complementary features of general and essential interest.

Traditionally, the largest share of meiotic research has been focused on DNA exchange and related features, whereas the immense field of protein–protein interactions in the rewiring of the meiotic cell out of and back into the mitotic cell cycle stood in second place. The concluding chapter of the preceding volume specifically deals with these meiotic aspects of molecular cell physiology (L. Pérez-Hidalgo, S. Moreno, and C. Martin-Castellanos, this SERIES). As pioneered with yeasts, genome-wide expression studies have started with identifying all the genes upregulated in meiotic cells and sorting them into functional categories. This is a long way off from knowing all their particular functions. To illustrate the scope of the barely charted field: of 4,824 annotated genes in *S. pombe*, 955 proteins contain *coiled-coil* motifs[4]; of these, 180 are upregulated before, during or after meiosis—21 exclusively so, but not expressed during mitosis (Ohtaka et al. 2007). The interactive potential of so many proteins is enormous, and the *systems biology of meiosis* has merely just begun.

To form a link between both books on *Recombination and Meiosis*, the list of chapter titles in the preceding volume is included after the Contents table of this book. In fact, as some of the individual chapters already had been published Online First, before the editorial decision to divide the printed edition into two books, 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 no neglect but reflect an obligatory compromise necessitated by publishing all manuscripts OnlineFirst immediately

[4]Coiled-coil motifs often serve as extended dimerization domains, as found in many filament components or structural linker proteins.

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 focusing on meiotic recombination. 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 thriving for the highest level of expertship, comprehensiveness, and readability.

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Copenhagen, Richard Egel
Ladenburg, April 2008 Dirk-Henner Lankenau

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Note Added in Proof

In the field of homologous recombination mechanisms, a recent experimental publication stands out as a very important breakthrough paper. Chen, Yang & Pavletich (2008) report crystal structures of RecA microfilaments, comprising five to six interconnected RecA moieties with single-stranded (presynaptic) or heteroduplex (postsynaptic) DNA. The structural coordinates confine the general considerations discussed in Chantal Prévost's chapter to the particular model suggested earlier by Prévost and Takahashi (2003). (i) The RecA-bound presynaptic ssDNA resembles B-form DNA in base-stacked blocks of three nucleotides per RecA subunit, where base stacking is interrupted towards the adjacent triplets. (ii) The ssDNA is bound from the backbone by two flexible

loops L1 and L2 of RecA. The L2 hairpins, in particular, fill in the unstacked space between the adjacent base triplets. (iii) The Watson-Crick edges are freely exposed to the solvent and ready for base pairing with a complementary strand. (iv) Heteroduplex formation with a second strand changes conformation of the primary strand only very little, and the complementary strand is held in position by Watson-Crick base pairing in B-form overall topology, actually with very few protein contacts to RecA. (v) By inference, the stretching-induced disruption of base stacking in the incoming donor duplex likely represents the most important feature in the RecA-mediated strand-exchange reaction.

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Crossing-Over and Disjunction

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Evolution of Models of Homologous Recombination

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Abstract With the elucidation of the structure of DNA in 1953, it became possible to think in molecular terms about how recombination occurs and how it relates to the repair of DNA damage. Early molecular models, most notably the seminal model of Holliday in 1964, have been followed by a succession of other proposals to account for increasingly more detailed molecular biological information about the intermediates of recombination and for the results of more sophisticated genetic tests. Our current picture, far from definitive, includes several distinct mechanisms of DNA repair and recombination in both somatic and meiotic cells, based on the idea that most recombination is initiated by double-strand breaks.

Abbreviations

DSB	double-strand break
dHJ	double Holliday junction
BIR	break-induced replication
SDSA	synthesis-dependent strand annealing
PMS	post-meiotic segregation
Ab4 : 4	aberrant 4 : 4 segregation
SSA	single-strand annealing

1 Introduction

In humans and other vertebrates, the repair of DNA damage by homologous recombination is essential for life. In addition, recombination is essential for the proper segregation of chromosomes in meiosis and for the generation of genetic diversity. Moreover, defects in DNA repair by homologous recombination are strongly correlated with many types of human cancers. For all these reasons, as well as for the purely intellectual pleasure of understanding these processes, the development of molecular models to explain homologous recombination has been an exciting area of study. In this review I focus on mostly genetic results that have driven the construction of molecular models of recombination; however, these models have been increasingly influenced by our growing understanding of the biochemical properties of gene products required to carry out recombination. The reader seeking more details concerning the actions of recombination proteins is directed to many recent review articles (Aylon and Kupiec 2004; Cahill et al. 2006; Cox 2003; Haber

2006; Krogh and Symington 2004; Kuzminov 1999; Lusetti and Cox 2002; O'Driscoll and Jeggo 2006; Raji and Hartsuiker 2006; Sung and Klein 2006), including other chapters in this BOOK or the accompanying volume in this SERIES. This review is necessarily historical, but when recent insights help to understand certain concepts, time warps occur.

1.1 Prelude

Before there was an understanding that the chromosome consisted of DNA, there was a fascination with the mechanisms by which homologous chromosomes could undergo crossing-over. Early ideas emerged from studies in *Drosophila* and maize. Even before cytologically identifiable homologous chromosomes were used to establish definitively that genetic recombination was indeed accompanied by a reciprocal exchange of chromosome segments (Creighton and McClintock 1931; Stern 1931), there was speculation how recombination might take place. Janssens (1909) imagined that pairs of homologous chromosomes must break and join, but how such pairs of breaks could be made to ensure that the recombined chromosomes had not lost any genes was difficult to imagine. Belling (1933) instead suggested that the newly copied chromatids could have undergone exchange through some sort of copy-choice mechanism as new chromatids were generated.

In a remarkable essay, Muller (1922) focused on the "synaptic attraction" between homologous chromosomes, likening it to the assembly of a crystal—a prescient anticipation of base-pairing! How recombination might happen was suggested from Muller's studies of X-irradiated chromosomes, which established the idea that chromosome breaks could be joined in novel ways to produce chromosome rearrangements (Muller and Altenburg 1930). Irradiation could also lead to apparently reciprocal exchanges between homologous chromosomes in mitosis and there was therefore the possibility that meiotic recombination might occur by some sort of breaking and joining. The finding that crossovers arising in meiosis were distributed non-randomly along the chromosome, exhibiting crossover interference, suggested that the mechanism of exchange was highly regulated (Muller 1916; Sturtevant 1915).

By the time the DNA structure was elucidated, it became evident that understanding the molecular nature of the gene and its functions, including recombination, would come—also as predicted by Muller (1922)—from the study of unicellular organisms, first in both bacteria and bacteriophage and then in fungi. In fact, before DNA was known to be a double helix of base-paired strands, Hershey and Chase (1951) had seen clear evidence of a hybrid bacteriophage chromosome in which one recombinant chromosome could yield both mutant and wild-type offspring for a particular gene. About 2% of the individual phage arising from this cross, when plated on a bacterial lawn, gave mottled plaques, which Hershey and Chase interpreted as evidence that

the genetic material was "heterozygous" at that locus. With the realization in 1953 that DNA was a double helix, it was possible to interpret these "heterozygotes" as evidence of hybrid DNA, with one strand carrying one allele and the complementary strand carrying the other (Levinthal 1954).

The study of meiosis in fungi was stimulated by the advantages of being able to recover all four haploid products of meiosis, as each spore would germinate into a colony; thus all four DNA strands of two recombining homologous chromosomes would be recovered (Fig. 1). The first important insight that opened the way to investigate the mechanism of recombination was made

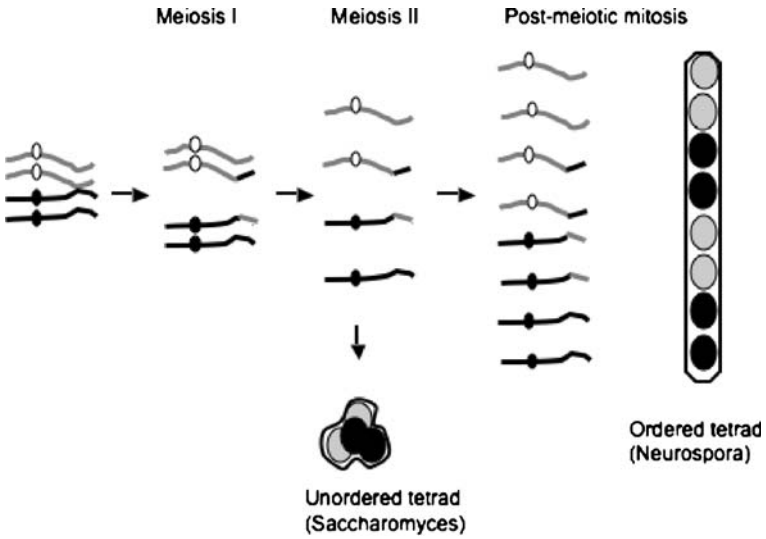

Fig. 1 Analysis of products of meiosis in ascospores. Following recombination at the 4-chromatid stage of meiosis, the four chromatids segregate, similar to what occurs in mammalian male meiosis. In budding yeast and other organisms with unordered tetrads the four nuclei are packaged into four spores within an ascus. Selective digestion of the ascus cell wall allows the micromanipulation of spores on an agar plate so that all four spores germinate. The resulting colonies can be scored for nutritional requirements, drug-resistance, growth at high temperature, and other attributes by replica plating them to different media or conditions. In *Neurospora* and other filamentous ascomycetes, there is a post-meiotic mitotic division, producing eight nuclei that are packaged into spores. In some organisms these asci are ordered, such that the position of the centromeres of each pair of homologous chromosomes are reflected in the linear order of the spores. Spore shape and spore color can be scored directly without microdissection and subsequent replica plating. A heterozygous marker (Aa) that has not undergone any crossing-over relative to its centromere will be seen as a first-division segregation (AAAAaaaa) pattern, whereas a meiosis in which there has been a single exchange between the marker and the centromere will have a second-division segregation pattern (AAaaAAaa). Gene conversions and post-meiotic segregations can be seen directly for visible markers in eight-spored ordered tetrads or after replica plating spore colonies to see sectored colonies

by Lindegren (1953), who found evidence of nonmendelian segregation of markers. Instead of always obtaining 2 wild-type: 2 mutant segregation for a carbon utilization gene, he found some tetrads with 3 : 1 or 1 : 3 patterns. To describe this phenomenon, Lindegren invoked the term *gene conversion*, first coined by Winkler in 1931 (Lindegren 1958). Gene conversions appeared to be *non-reciprocal* transfers of genetic information, very different from the reciprocal exchange events in crossing-over.

The primitive state of the *S. cerevisiae* genetic map precluded Lindegren from showing what had happened to nearby markers, but Mitchell (1955) studying *Neurospora* was able to show that while one marker was displaying nonmendelian segregation, flanking genetic markers segregated 2 : 2. Thus gene conversion was a local recombination event rather than a problem affecting an entire chromosome arm. Mitchell also noted that gene conversions and crossing-over in a small interval were correlated, and Freese (1957) went further to suggest that they were the consequence of a single event. An elegant proof that gene conversions were bona fide non-reciprocal transfers of the original alleles (rather than new mutations) was provided by Fogel and Mortimer (1969).

It took several more years before two other types of nonmendelian segregation pattern—post-meiotic segregation (PMS)—were appreciated. These were first seen in fungi in which meiosis was followed by a post-meiotic mitotic division prior to spore formation, leading to the ordered arrangement of 8 spores reflecting the orientation of the centromeres at the time of the first meiotic division. An ascus with no crossover or gene conversion between the marker and its centromere would give a "first division segregation" pattern (++ ++ −− −−); a crossover between the marker and its centromere yielded second division segregation (++ −− ++ −−). A 6 : 2 gene conversion appeared as (++ ++ ++ −−). Olive (1959) found the segregation of a gray-spore (*g*) allele of *Sordaria* included not only 4 : 4 and both 6 : 2 and 2 : 6 asci (i.e., those expected for gene conversion) but also asci with 5 : 3 and 3 : 5 segregation, in which one meiotic product had given rise to one mitotic copy with the *g* allele and the other with *G* (i.e., ++ ++ +− −−). These outcomes were reminiscent of the "heterozygous" results in bacteriophage crosses. Subsequently Kitani et al. (1962) found the last important nonmendelian segregation pattern of so-called aberrant 4 : 4 (Ab4 : 4) asci (++ +− −+ −−).

Kitani et al. (1962) also made another fundamentally important observation. Among asci that exhibited 6 : 2, 2 : 6, 5 : 3, 3 : 5 or Ab4 : 4 segregation, about 36% had also undergone a reciprocal crossing-over between adjacent markers that flanked the aberrantly segregating *g* locus. In contrast, among all tetrads the two markers showed only 4% crossing-over. Moreover, in almost all of the cases, a chromatid that exhibited PMS was one of the two chromatids involved in the crossover event. These observations suggested that crossing-over and these nonmendelian segregation events were intimately connected, and that the process of crossing-over often generated heterodu-

plex DNA. A similar conclusion was reached by Fogel and Hurst (1967); in budding yeast, with four spores, the appearance of 5 : 3 and 3 : 5 types could be seen by careful replica plating of the original spore colonies such that one half of the colony would be wild-type and the other half auxotrophic for some nutritional marker. Consequently, budding yeast data are also discussed in terms of 8 DNA strands.

1.2 The First Molecular Models of Recombination

Several early models imagined that gene conversions arose by template switching during the pre-meiotic replication of homologous chromosomes (Freese 1957; Lissouba et al. 1962; Stadler and Towe 1963). Although these "switch" or "copy-error" models could account for gene conversion and crossing-over, they did not offer explanations of PMS outcomes. One influential model, based on density analysis of recombinant bacteriophage, was the "copy-choice" mechanism proposed by Matthew Meselson and Jean Weigle (1961). Their model suggested that the end of a broken molecule could be unwound and that each strand of a broken chromosome end could base-pair with complementary sequences of an intact DNA duplex. Strand pairing then promotes copying of the template, producing a nonreciprocal crossover product (Fig. 2). This model contained apparently the first representation of the 4-strand branched intermediate now called a Holliday junction (HJ). We will return to ideas about break-copy recombination near the end of the review, when we examine mechanisms of recombination-dependent DNA replication, also known as break-induced replication.

Break-copy ideas were almost immediately confronted with data supporting break-join recombination. In the same year that Meselson and Weigle proposed break-copy, Kellenberger et al. (1961) used density-gradient analysis of phage λ parents of different densities, combined with ^{32}P labeling of one parent to show that most recombination involved a physical exchange of DNA with little new synthesis (Anraku and Tomizawa 1965).

In 1962, Robin Holliday (1962) briefly speculated that recombination might involve junctions of parental DNA molecules that contained heteroduplex DNA. Moreover, extrapolating from recent findings of template-directed repair of UV-induced lesions, Holliday conjured up the idea that mismatches in heteroduplex DNA could be repaired in a somewhat analogous fashion. Such repair, he noted, could account for gene conversions.

Soon after, Harold Whitehouse (Whitehouse 1963) provided the first illustrated molecular models that would use heteroduplex DNA to create a reciprocal exchange between two DNA molecules. Whitehouse suggested two variations of his model (Fig. 3). In both cases he suggested that single-strand DNA breaks could occur in adjacent DNA molecules, either at different points (Fig. 3A) or at the same point (Fig. 3B), but in strands of opposite polar-

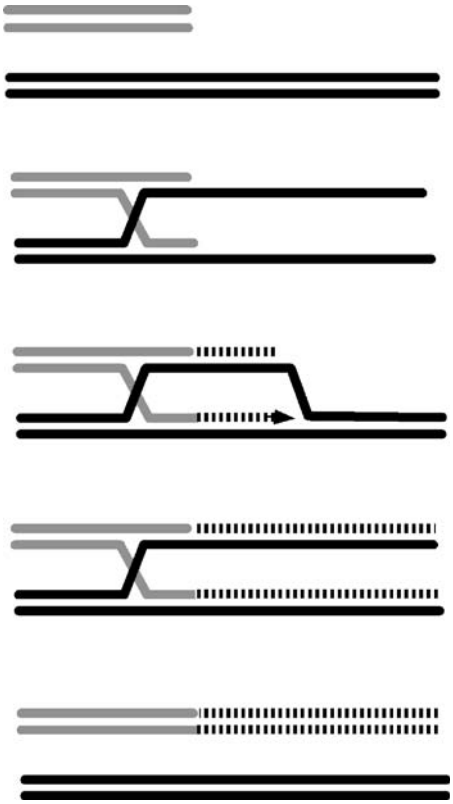

Fig. 2 Meselson and Weigle's 1961 Break-Copy recombination mechanism. The two strands of a broken chromosome fragment can form base pairs with an intact template and promote copying to the end of the template, thus producing a recombined, full-length product

ity. In the first model, the nicked single strands could unwind and pair together to form hybrid (heteroduplex) DNA. Subsequently the gaps created by the formation of the heteroduplex could be filled in by new DNA synthesis. Whitehouse then suggested that there would be "another cycle of strand separation and hybridization, degradation of surplus DNA, and finally correction of mismatched base pairs." In the second model (Fig. 3B), each of the initially displaced strands would pair with a newly copied version of the opposite homolog, again creating regions of heteroduplex DNA at the crossover point. The last step involved the removal of part of two "old" strands of DNA to complete the crossover structure. The heteroduplex regions could then be subject to some type of repair of mismatches to account for various nonmendelian ratios of alleles among the meiotic products. During the completion of the recombination event, there were additional patches of new synthesis; these could yield gene conversion events without being directly associated with a crossover.

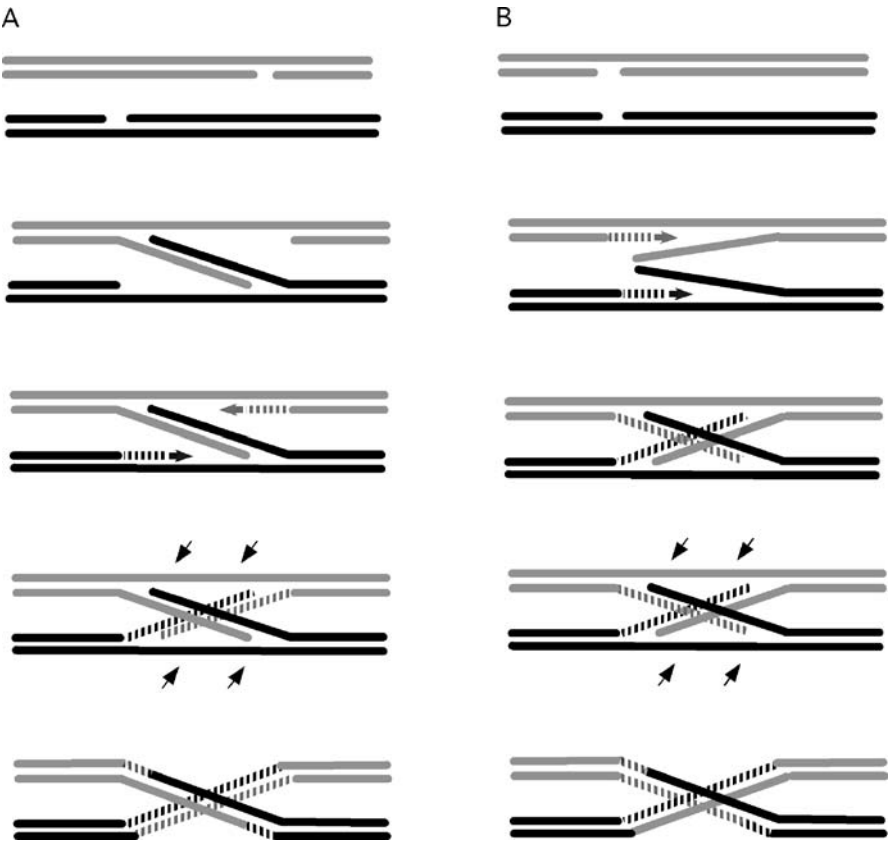

Fig. 3 Whitehouse's 1963 models. **A** Nicks at different locations in strands of opposite polarity allowed annealing and joining of two DNA molecules by a region of heteroduplex DNA. New DNA synthesis, strand displacement and annealing creates a second cross-connection, again with heteroduplex DNA. The "extra" strand of DNA is excised and degraded (indicated by *arrows*), leaving a crossover. Completion of DNA synthesis to join all strands results in flanking regions in which there are 3 strands of one parental type, allowing gene conversions to be made without an immediate crossover. **B** A similar process involving strands of the same polarity and where the nicks occur at the same position. Here heteroduplex is formed between old and newly synthesized strands

2 Robin Holliday's Remarkable Model

Robin Holliday's 1964 model (Holliday 1964) created a much simpler and elegant molecular view of recombination that accounted for all of the key findings made by his predecessors. Holliday envisioned that crossing-over began with a coordinated pair of single-strand nicks—but on strands of the same polarity—on a pair of homologous chromosomes. These nicked strands

could be unwound and displaced, allowing an exchange of single strands, accounting for the formation of regions of heteroduplex DNA that might cover a region where the DNA differed between the homologous chromosomes (Fig. 4). This reciprocal exchange of single DNA strands led to the creation of

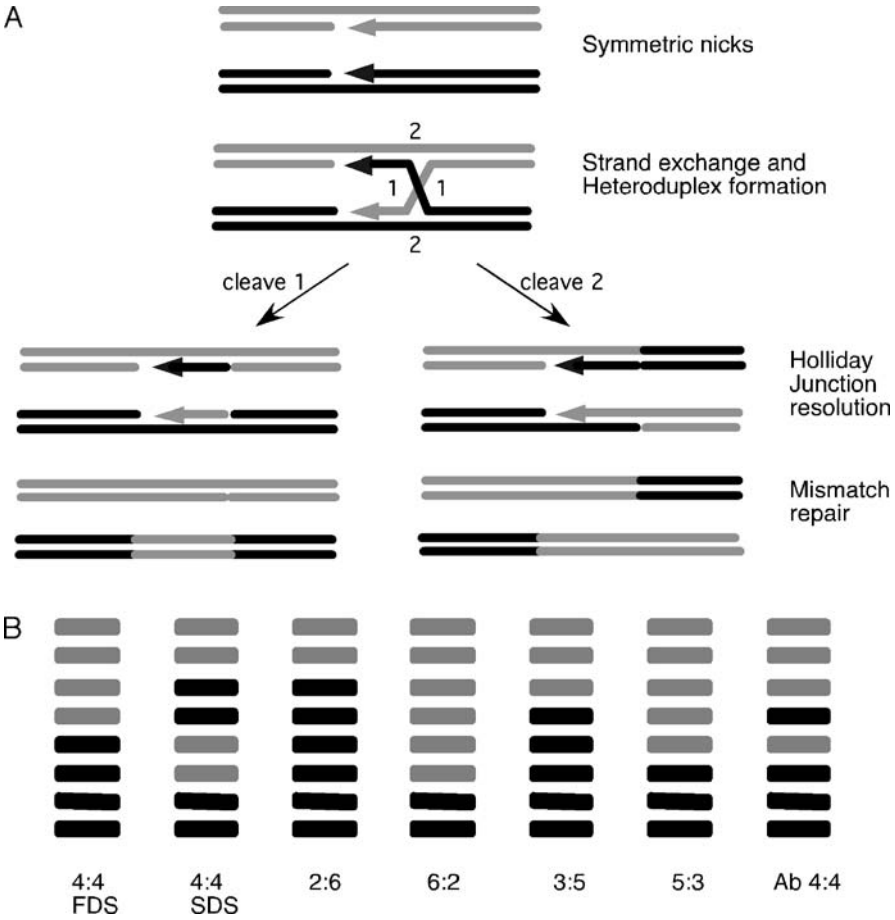

Fig. 4 Holliday's 1964 model. **A** A pair of nonsister chromatids after meiotic DNA replication are shown; the two other chromatids, uninvolved in recombination, are not shown. A pair of same-strand nicks leads to a reciprocal exchange and formation of symmetric heteroduplex connected by a 4-stranded symmetric structure now known as a Holliday junction (HJ). The HJ can be cleaved by cutting either of two pairs of strands (orientations 1 and 2). Crossovers occur when the HJ is cleaved so that only the crossing-strands connect the two homologous chromosomes. In the example shown, mismatch corrections lead to a 6 : 2 gene conversion. **B** Heteroduplex regions can be converted, restored or left unchanged depending on the efficiency of mismatch correction. All types of nonmendelian segregation patterns can be accounted for by this mechanism, as shown here for an ordered tetrad

the four-stranded structure—what we now call a Holliday junction—which could be resolved to give both crossover and noncrossover outcomes. The second key idea, drawn from his 1962 speculations, was that mismatch repair of heteroduplex DNA could produce aberrant ratios of alleles among the progeny, including both gene conversions and post-meiotic segregations (Fig. 4B).

Combining the idea that Holliday junctions could be resolved either with or without crossing-over with the idea that heteroduplex intermediates could be restored, converted or left unrepaired, Holliday set out a mechanism that accounted for all of the results obtained in various fungal systems. Over time, however, as more data accumulated, it became clear that—in detail – the proportions of various outcomes expected from Holliday's model did not fit the observed types of tetrads recovered from several different fungi. Consequently, Holliday's model has undergone several important evolutionary modifications that will be discussed below, but the three ideas that he emphasized—the creation of heteroduplex DNA by the exchange of a single strand of DNA, the formation of a branched intermediate Holliday junction and the mismatch correction of heteroduplex DNA—remain the foundation of our present understanding.

2.1 Strand Exchange by Single-Strand Annealing

Soon after Holliday's model appeared, Charles Thomas (1966) offered a slightly different view in which all of the outcomes would be linked to reciprocal crossing-over (Fig. 5A). In Thomas' model, staggered nicks would occur on both strands of each duplex molecule and the separation of strands would permit the formation of reciprocally recombined molecules, linked by regions of heteroduplex DNA. This mechanism of *single-strand annealing* (SSA) could work even if all the nicks were not at precisely the same position, because gaps or overhanging single-stranded segments could be enzymatically filled in or clipped off, respectively. We will return to a discussion of SSA towards the end of the review, but in the case where SSA occurs following a double-strand break.

2.2 Evidence Favoring Holliday's Model: Hotspots and Gradients of Gene Conversion

Evidence supporting several features of Holliday's model came from more intensive analysis of gene conversion events within individual genes. In the ascomycete *Ascobolus immersus* Jean-Luc Rossignol and his colleagues had isolated many alleles within genes affecting spore color (Rossignol 1969). Some alleles showed a high rate of nonmendelian segregation, with as many a 5% of the asci containing a gene conversion; other alleles had conversion

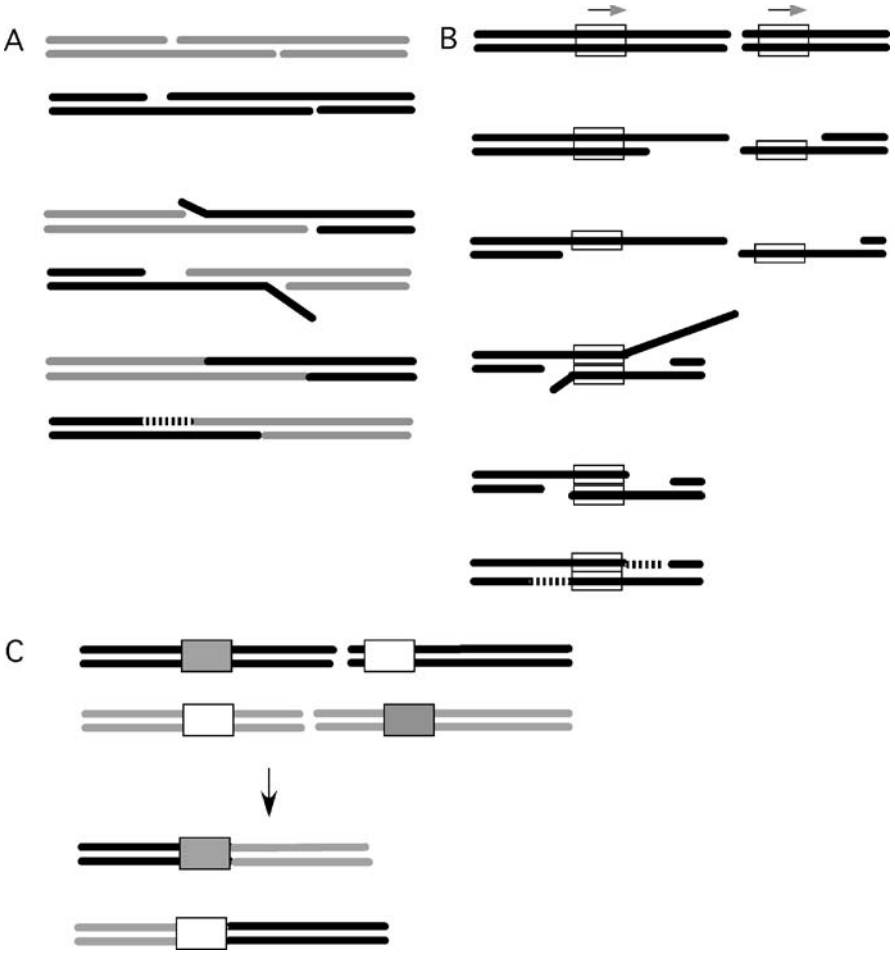

Fig. 5 Single-strand annealing. **A** Charles Thomas' SSA model to obtain reciprocal recombination by annealing overlapping single-strands of DNA from two chromosomes with offset nicks on both strands. **B** DSB-induced SSA leading to an intrachromosomal deletion between directly oriented, non-tandem repeats. The DSB ends are resected by 5′ to 3′ exonucleases and Rad52-mediated annealing between flanking homologous sequences can occur, even in the absence of Rad51. Long 3′ ended ssDNA tails can be cleaved off and the missing DNA filled in by using the 3′ ends of the paired strands as primers. **C** Reciprocal crossovers (translocations) created by SSA can be accomplished if there are a pair of DSBs flanking pairs of homologous sequences

rates 10 times lower. When the rate of nonmendelian segregations of each allele, crossed to wild-type, was plotted versus the position of each allele within the gene, it became apparent that there was a distinct gradient, with most alleles showing high levels of nonmendelian segregation at one end (Lissouba et al. 1962; Rossignol 1969).

As more alleles were obtained it became clear that some high-frequency gene conversion alleles yielded primarily 6 : 2 or 2 : 6 patterns whereas other alleles gave 5 : 3 and 3 : 5 patterns, with some 6 : 2 and 2 : 6 (Leblon 1972a,b). Similarly there were both types among infrequently converting alleles. A similar conclusion was reached for alleles of the *arg4* locus in *S. cerevisiae* (Fogel et al. 1979; Mortimer and Fogel 1974)[1].

The gradient of gene conversion along a gene could be explained if there were a *hotspot*—a preferential site of initiation of the recombination—at one end of the gene. This could be the site of DNA strand cleavage. The probability that heteroduplex DNA formation resulting from strand exchange would include an allele within the gene would be roughly proportional to the distance between the hotspot and the allele. Thus the probability that nonmendelian segregation would occur would also be proportional to the distance of the allele from the site of initiation of recombination.

2.3 Challenges to the Holliday Model

The Holliday model provided a conceptual basis for understanding the kinds of tetrads that arose in various fungi and was completely consistent with what little was known about recombination in higher organisms, but further analysis of fungal genetic data began to present examples where the observed patterns of segregation were inconsistent with the outcomes expected from Holliday's model. There were two major concerns. First, whereas Holliday's model imagined *symmetric heteroduplex* DNA (that is, where both chromatids involved in the recombination event form equivalent heteroduplex DNA), the data reviewed below were more consistent with a recombination intermediate that had only one heteroduplex region (that is, *asymmetric heteroduplex*). Second, Holliday's model suggested that all the crossover events should be located at the end of the heteroduplex DNA opposite from the point where the strands were nicked and unwound. This, too, proved not always to be the case.

2.4 The 5 : 3 Paradox

In Holliday's strand exchange model, the most frequent types of nonmendelian segregations are 6 : 2 and 2 : 6 gene conversions that would be expected if one heteroduplex region was converted and the other was restored to its initial genotype. This suggests that in general conversion and restoration are equally likely to occur. Now consider 5 : 3 tetrads in *Neurospora* or

[1] During this period that recombination models were being developed, their authors took into account recent experimental findings that had been presented and discussed at meetings long before they made their way into print—in contrast to current practice where data are often only presented at meetings if they are in press or published.