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Multi-author Reviews

Homologous recombination

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Introduction

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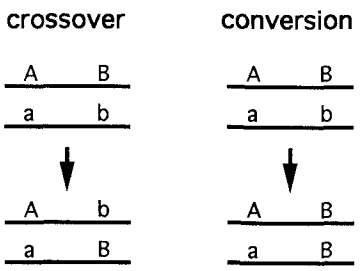
Genetic recombination is the molecular process by which new combinations of the genetic material are generated. The ubiquitous occurrence of this process in living organisms is indicative of its biological significance. It was recognized early that recombination (besides mutation) contributes to Darwinian evolution by providing the raw material for natural selection. However, recombination is also important for the individual. It provides a pathway for the repair of damaged DNA, and in eukaryotes recombination is essential for proper chromosome segregation in meiosis. Recombination processes may lead to oncogene activation or loss of tumor suppressor genes, important steps in carcinogenesis. And lastly, recombination is a useful tool in molecular genetics for applied and fundamental research. These aspects, together with the intellectual challenge they pose, will hopefully guide the reader through this issue.

Genetic recombination that requires extensive sequence homology in the participating DNA molecules is generally classified as homologous recombination. Various types of homologous recombination can be defined depending on the location of the homologous sequences that interact with each other (allelic positions on homologous chromosomes, on sister chromatids, within the same chromatid, on heterologous chromosomes). A coherent terminology, as proposed by Petes and Hill[1], could serve to avoid the present confusion. A second class of events can be summarized as site-specific recombination where recombination occurs at specific DNA sequences that share little to no homology. These events include inversion of chromosomal segments, insertion and excision of viruses, and the rearrangements that form functional immunoglobulin genes. A third class is transposition by which DNA sequences are moved from one place in a genome to other locations in the same or a different genome in more or less random fashion. Illegitimate recombination is the fourth class of events in which neither sequence homology nor a specific site nor activities of a defined genetic element can be identified. These events can lead to insertions, deletions, and DNA rearrangements that pose great practical problems for the genetic manipulation in somatic cells of higher organisms where illegitimate recombination is far more frequent than homologous recombination.

Homologous recombination events can be divided at the phenomenological level into two classes, crossover and conversion (see figure).



Crossover recombination is the reciprocal exchange of DNA sequences. Conversion is the non-reciprocal transfer of genetic information. Both types, crossover and conversion, are equally important, although many population biologists apparently disagree, and ignore the existence of conversion[2]. The distinction between crossover and conversion can only be made when both substrates and *both* products of an event can be analyzed. Analysis of one recombination product, usually the one selected for, does not allow a distinction. As shown by the figure, the recombinant chromosome (a B) is the same for both crossover and conversion. This recovery of both recombination products is not trivial in most systems, including *Escherichia coli*. It is readily achieved in meiotic recombination in fungi as all four products of one meiosis can be individually recovered and analyzed. This specific reason besides many other well known experimental advantages has propelled yeasts as the prime eukaryotic model systems for the study of homologous recombination.

The purpose of this issue is to place some areas of homologous recombination research into the spotlight. We have tried to identify those areas where fascinating advances have been achieved recently and we have also included topics where recombination might be related to other biological phenomena. Nevertheless, the choice is subjective. Some topics like ectopic recombination, recombination mutants, or recombination and gene targeting in mammalian cells are missing due to the space limitations and other reasons. Another important topic (mating-type switching in the yeasts *S. cerevisiae* and *S. pombe*) is missing because Tarmo Ruusala (Uppsala University) succumbed to illness and to our sorrow passed away last fall at the age of 40.

The articles presented here illustrate the diversity of approaches used to study homologous recombination, such as formal genetics, molecular genetics, biochemistry, biophysics, and cytology. The most spectacular progress has been made in *E. coli*. Thanks to the pioneering work of John Clark[3] a large collection of recombination genes has been identified. Cloning and sequencing these genes has provided the means to overexpress, purify, and characterize the respective proteins. Now this approach has paid off as can be seen in the first three contributions. Stasiak and Egelman highlight the *E. coli* RecA protein which is central to most recombination in that organism. RecA is serving as a paradigm for understanding the formation of hybrid (or heteroduplex) DNA, a central intermediate in homologous recombination. The second article by Kowalczykowski illustrates one road towards the reconstitution of *E. coli* homologous recombination in the test tube. By combining RecA, RecBCD, and SSB the early steps of the major recombination pathway in *E. coli* have been reconstructed. Another path towards the same goal is described by Müller and West, reconstructing with RuvABC the late steps of homologous recombination: the resolution of the Holliday junction, another central intermediate. Both approaches have met in the middle and are ready to be combined. The understanding of these processes in eukaryotes is lagging far behind. The approaches to understanding hybrid DNA formation in eukaryotes are discussed by Heyer. In all eukaryotes examined, structural homologs of the *E. coli* RecA protein have been identified and yeast genetics suggests that they might be important recombination proteins like their prokaryotic counterparts. Homologous recombination does not occur with equal likelihood throughout the chromosomes. Some areas are hot and others are cold for recombination. Much can be learned (especially about initiation of recombination) from analyzing so-called hotspots of recombination, as shown in the article by Smith. Nonrandom distribution of recombination events is also evident within individual genes as demonstrated by the polarity gradients in gene conversion in fungi. Detailed analysis of this phenomenon starts to reveal fundamental insights into the recombination machinery. The intellectual dispute between Petes and Nicolas concerning polarity also gives testimony to the continuous modification of recombination models based on data from yeast genetics. For a long time it has been attempted to fit all data from fungi into a unified model for meiotic recombination. It is likely that this will turn out to be impossible and that different pathways will be described within and between species. The contribution of base mismatch repair to the fidelity of DNA replication is well documented. Fox, Radicella, and Yamamoto show how a surprising multitude of mismatch repair systems are affecting homologous recombination in *E. coli*. Undoubtedly, the situation in eukaryotes will not be less complex. Other DNA metabolic processes may interfere with recombination as they work on the same DNA substrate. Gangloff, Lieber, and Rothstein discuss the consequences that transcription and topoisomerases have on genetic recombination by altering the DNA topology. This connection might have been used by cells with increasingly higher DNA content to confine recombination to structural genes where it matters the most[4]. The prime model systems to study recombination to date are *E. coli* and yeasts. The ease of their genetic manipulation and the high levels of homologous recombination (versus low levels of illegitimate recombination) are among the obvious reasons. However, not everything can be learned from these systems and not all generalizations derived from them may hold. Bezzubova and Buerstedde present an interesting model for homologous recombination in vertebrates, the chicken immunoglobulin system. The almost yeast-like reverse genetics possible in this system bears much promise. The study of genetic recombination in plants is still in the early stages, as comprehensively reviewed by Puchta, Swoboda, and Hohn. The importance of developing recombination as a molecular tool in plant gene manipulation will certainly push this field of study forward. Homologous recombination shuffles the genetic composition of the genomes but might also be involved in other biological phenomena. Loidl discusses the relation between homologous recombination and the cytological structures of meiosis. The eukaryotes have developed a highly complex (and in molecular terms largely not understood) mechanism for fully pairing two homologous and replicated genomes, catalyzing high frequency of recombination, and subsequently assuring proper segregation of the recombined chromosomes into four haploid gametes. Following this general review of meiosis Kohli and Bähler present the analysis of recombination and meiosis in the fission yeast *Schizosaccharomyces pombe* which differs in some important details from other eukaroytes. By comparison of both yeasts, *S. cerevisiae* and *S. pombe*, the fundamentally conserved features can be elucidated and then

extrapolated to higher organisms. Finally, Rossignol and Faugeron enrich the wide spectrum of contributions on homologous recombination with their discussion of the gene inactivation of repeated genes in some organisms. This is another phenomenon requiring the molecular pairing of homologous DNA sequences.

Readers wishing to learn more about recombination are referred to some excellent books on this topic. A classic with a more formal treatment of the topic has been written by Stahl[5]. Books with collections of up-to-date articles covering far more aspects of genetic recombination than we could attempt here have appeared recently[6–8]. We thank all the authors for their contributions and for making the assembly of this review an intellectually stimulating experience. We also thank Managing Editor Dr. Holger von Hahn and the Birkhäuser Verlag for their efficient production of this volume. We both dedicate our contributions to Prof. Urs Leupold on the occasion of his seventieth birthday; Urs Leupold has pioneered the study of homologous recombination in Switzerland and he inspired us with his fascination for this field of biological research.

1 Petes, T. D., and Hill, C. W., Recombination between repeated genes in microorganisms. A. Rev. Genet. *22* (1988) 147–168.
2 Gutz, H., and Leslie, J. F., Gene conversion: a hitherto overlooked parameter in population genetics. Genetics *83* (1976) 861–866.
3 Clark, A. J., and Margulies, A., Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc. natl Acad. Sci. USA *53* (1965) 451–459.
4 Thuriaux, P., Is recombination confined to structural genes on the eukaryotic genome? Nature *268* (1977) 460–462.
5 Stahl, F. W., Genetic recombination. W. H. Freeman and Company, San Francisco 1979.
6 Kucherlapati, R., and Smith, G. R. (eds), Genetic Recombination. American Society for Microbiology, Washington, D.C. 1988.
7 Low, K. B. (ed.), The Recombination of Genetic Material. Academic Press, Inc. 1988.
8 Gottesman, M. E., and Vogel, H. J. (eds), Mechanisms of Eukaryotic DNA Recombination. Academic Press, Inc. 1992

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