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genome stability

DNA repair and recombination

James E. Haber

Genome Stability
DNA Repair and Recombination

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Preface
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Summary: "Genome Stability: DNA Repair and Recombination describes the various mechanisms of repairing DNA damage by recombination, most notably the repair of chromosomal breaks. The text presents a definitive history of the evolution of molecular models of DNA repair, emphasizing current research. The book introduces the central players in recombination. An overview of the four major pathways of homologous recombinational repair is followed by a description of the several mechanisms of nonhomologous end-joining. Designed as a textbook for advanced undergraduate and graduate students with a molecular biology and genetics background, researchers and practitioners, especially in cancer biology, will also appreciate the book as a reference"--Provided by publisher.
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Genome Stability
DNA Repair and Recombination

For Melissa, Deborah, and Susan

PREFACE

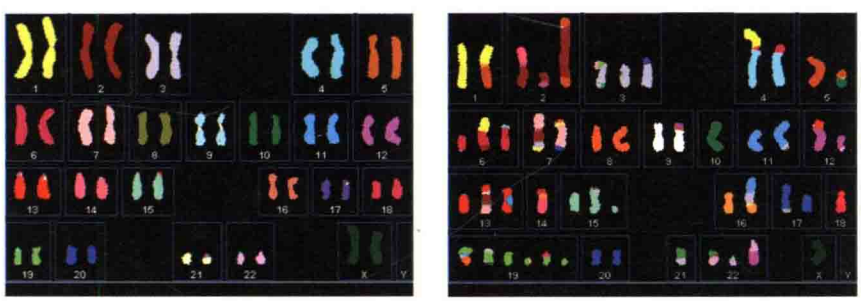
The primordial tumorigenic cell [...] is, according to my hypothesis, a cell that harbours a specific faulty assembly of chromosomes as a consequence of an abnormal event.

Theodore Boveri (1914)
Translated by Henry Harris

The factors responsible for fusions of broken ends or for the healing of a broken end are not understood but are probably related to the method by which the chromosome becomes broken and to the physiological conditions surrounding the broken end.

Barbara McClintock (1941)

One of the most striking molecular aspects of cancers cells is their shocking departure from the normal chromosome number and arrangement. DNA replication is over 99% accurate, but the task of replicating six billion base pairs of human DNA in every cell is still precarious, both in terms of simple mutations and—more dangerously—in the creation of double-strand breaks (DSBs) that must be repaired. This textbook explains how genome stability is maintained.



In contrast to normal chromosomes (above left), chromosomes from tumor cells (above right) exhibit dozens of alterations—truncations, translocations, duplications, and amplifications of chromosome segments, as well as gains and losses of whole chromosomes.

Cells have evolved two key processes to deal with broken chromosomes. First, they have elaborated a variety of different mechanisms to repair these breaks, most often using an intact sister chromatid or an homologous chromosome as the template to patch up the break. Much of this book will deal with understanding in detail how these largely error-free repair mechanisms function. These *homologous recombination* mechanisms are backed up by other, less precise *nonhomologous end-joining* pathways that can join broken ends together, with little regard for their origin. When the more accurate DNA repair processes fail, these alternative mechanisms take over, creating the rearrangements that we see in tumor cells. Repair of chromosome breaks is enhanced by a second process, termed the DNA damage checkpoint, which operates initially to prevent cells with chromosome breaks from entering mitosis, thus providing more time for repair to take place. If this restraint fails,

then a second aspect of the DNA damage checkpoint is to destroy cells with unrepaired DNA damage by triggering apoptosis. Nearly all tumor cells have lost their ability to repair DSBs by homologous recombination and/or have lost the DNA damage checkpoint response.

Two exceptionally thoughtful books initially influenced my own thinking and prompted my wish to contribute a more molecular perspective. The first is H.L.K. Whitehouse's suggestive *Towards an Understanding of the Mechanism of Heredity* (1969); the second is Frank Stahl's inventive *Genetic Recombination: Thinking About It in Phage and Fungi* (1979). Both of these books preceded the explosion of molecular biological and genetic techniques that have made it possible to dissect the mechanisms of DNA repair in great detail, most especially in bacteria and yeasts, but increasingly in metazoans. I have jokingly said that this textbook is the sequel to Stahl's, but "thinking about it in fungi and mice." I have included a number of examples and concepts derived from studies of bacterial recombination and a smaller number from the emerging world of Archaea, but the focus is on eukaryotic chromosomes and their repair and recombination. Much of this textbook concentrates on chromosomal DSBs, the most dangerous type of DNA lesions. Some types of DNA repair—nucleotide excision repair or base-excision repair—are mentioned tangentially, but the focus is on repairing a completely broken chromosome.

This text is for advanced undergraduate and graduate students in molecular biology, genetics, and biochemistry. It is also intended as a reference for researchers and practitioners, especially in cancer biology. In writing this textbook I have assumed that the reader will have had some basic knowledge of genetics and molecular biology, knowing roughly how DNA replication proceeds. Consequently, the book begins with the problem of re-starting DNA replication at sites of damage or breakage (Chapter 1), as a way of introducing some of the basic mechanisms that are revisited in more detail in later chapters. The focus is on homologous recombination, driven by RecA and Rad51 recombination proteins, but Chapter 15 addresses nonhomologous end-joining in its several guises. After an overview of the various DSB repair mechanisms in Chapter 2, we begin with a review of the key recombination proteins RecA and Rad51 and how they work (Chapter 3). Then we turn to how DNA ends are processed to enable recombinase proteins to be loaded and to begin the search for homologous sequences with which repair can be effected (Chapter 4). Chapters 5 through 9 deal with different types of homologous recombination to repair a broken chromosome: single-strand annealing, mitotic gene conversion, and break-induced replication. A mixture of genetic and molecular biological evidence is presented to support our current understanding of the molecular mechanisms that underlie these processes. But homologous recombination is also a tool in modern genetics, so Chapters 10 and 11 examine gene targeting and site-specific recombination in detail. Only then do we confront recombination as it was initially studied a century ago—in meiosis (Chapters 12 and 13)—because meiotic recombination has elaborated and differentiated the basic mechanisms of DSB repair to ensure the accurate completion of generating recombined haploid germ cells from a diploid.

I have been forced to choose among many experiments to illustrate the important concepts in the book and have not mentioned numerous critical findings that led up to the selected experiments. Each of these experiments is cited in the relevant figure legends and each chapter includes suggested reading. Many other possible citations are absent, but they are available to the reader in two ways. First, I have added as an Appendix to this book (available online) a history of the evolution of molecular mechanisms of recombination, which has about 250 references that give full credit to the brave pioneers who launched the studies we continue today. Second, a combination of PubMed and Google searches will quickly bring an interested student to the relevant literature. Sprinkled throughout the book are brief boxes on nomenclature, perspectives, and measurement. The book also contains over 300 images and illustrations that, I hope, provide a way to visualize the processes that occur inside the human cell, a world too small to see.

ONLINE RESOURCES FOR STUDENTS AND INSTRUCTORS

Accessible from www.garlandscience.com/genomestability, the Student and Instructor Resource Websites provide learning and teaching tools created for *Genome Stability*. This book presents molecular models of recombination based on our present understanding, reflecting genetic, molecular biological, and biochemical approaches. But these models slowly evolved from ideas that first emerged soon after the discovery of the structure of DNA. Many clever ideas were postulated by creative scientists whose fundamentally important contributions are often overlooked as we focus on our present knowledge of these processes. An historical account of the way that our present models of DSB-mediated recombination evolved is presented in an Appendix in PDF format entitled "Evolution of Models of Homologous Recombination." This Appendix is available on both the Student and Instructor Resource Sites.

For Students: The Student Resource Site is open to everyone, and users have the option to register in order to use book-marking and note-taking tools.

For Instructors: All of the images from the book are available in two convenient formats: Microsoft PowerPoint® and JPEG. They have been optimized for display on a computer. Figures are searchable by figure number, figure name, or by keywords used in the figure legend from the book. The Instructor Resource Site requires registration; and access is available to instructors who have assigned the book to their course. To access the Instructor Resource Site, please contact your local sales representative or e-mail science@garland.com. You can also access the resources available for other Garland Science titles.

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I could not have even imagined writing a book on DNA repair and recombination without the invaluable help of many people. Jeffrey Hall taught me much of what I know about classical *Drosophila* genetics and I am especially grateful to Barbara McClintock who encouraged me early on in our discussions of her studies of genome instability in maize. A visit by Isamu Takano to Harlyn Halvorson's lab, coupled with my own interest in how mating type genes controlled meiosis and gene expression in yeast, prompted my investigation of homothallic *MAT* switching. Innumerable conversations with colleagues all over the world and collaborations with more than 75 different labs have also contributed to this endeavor. I owe particular thanks to my role models and colleagues, some of whom also reviewed individual chapters: Maury Fox, Frank Stahl, Jean-Luc Rossignol, Francis Fabre, Matt Meselson, Yasuji Oshima, Jack Szostak, Rod Rothstein, Richard Kolodner, Scott Hawley, Tom Petes, Shirleen Roeder, Nancy Kleckner, Maria Jasin, Scott Keeney, Neil Hunter, Doug Bishop, Michael Lichten, Dana Carroll, Bill Holloman, Lorraine Symington, Bill Holloman, Phil Hastings, Susan Rosenberg, Susan Lovett, John Wilson, Tony Carr, Benoit Arcangioli, Bernard Dujon, Steve West, Steve Kowalczykowski, Michael Cox, Hannah Klein, Patrick Sung, Akira Shinohara, Fred Alt, Shunichi Takeda, Ted Weinert, Virginia Zakian, Gerry Smith, Wolf Heyer, Simon Boulton, Roland Kanaar, Bernard Lopez, Vincenzo Costanzo, Ralph Scully, Marco Foiani, Doug Koshland, Angelika Amon, and Titia de Lange. I am certain I have forgotten to mention others and apologize in advance. I have deep, abiding memories of Nick Cozzarelli, Seymour Fogel, Fred Sherman, Ira Herskowitz, and my mentors Dan Koshland, Harlyn Halvorson, and Alan Wilson.

Most especially my understanding of DSB repair has come from the day-to-day discussions and the exceptional creativity of more than 80 postdocs and graduate students (plus several devoted technicians and a number of undergraduates) with whom I have been blessed to work. Here is a list of the people from my own lab with whom I have so far published: Peter Wejksnora (my first grad student), Paloma Liras (first postdoc), Sim Gek Kee (first undergrad), Ellen Kraig, Susan Remer, Nancy Pearson, Anne Comeau, Deborah Wygal Mascioli, Dave Rogers, Sue Stewart, Barbara Garvik, Jeanne George, Pat Thorburn, Elaine Sugarman, Norah Rudin,

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I first started working on a version of this book as a John Simon Guggenheim Fellow in 2000, but that effort soon faltered as I realized how little we really knew about the mechanism of strand exchange, the resolution of Holliday junctions, and much more. However the following decade has been remarkable in unveiling many of these mysteries. I didn't resume serious work on this book until I was a Fellow of the Radcliffe Institute for Advanced Study in 2008. Sabbatical visits with Steve West (London Research Institute, UK), Nevan Krogan (University of California, San Francisco), and Geneviéve Almouzni (Institut Curie) moved things along. Brandeis University has been a wonderful home for over 40 years and I have had the benefit of teaching bright students and working with stimulating faculty colleagues. I am particularly indebted to Susan Lovett, my DNA repair colleague, and to Jeffrey Hall, who—when I first started to teach—introduced me to the world of *Drosophila* and maize genetics, to chromosome segregations, and especially to the work of Barbara McClintock and other pioneers in the study of chromosome instability. Research from my own lab has been generously supported by the National Institutes of Health, the National Science Foundation, the US Department of Energy, and the American Cancer Society.

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