

genome stability

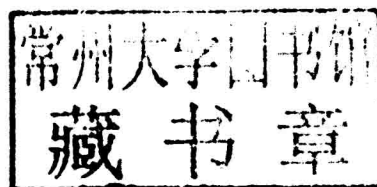
DNA repair and recombination

James E. Haber

Genome Stability

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Preface

Images of normal chromosomes and those from tumor cells courtesy of Molecular Cytogenetics of Common Epithelial Cancers, Cancer Genomics Program, University of Cambridge. With permission of Paul Edwards, University of Cambridge.

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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

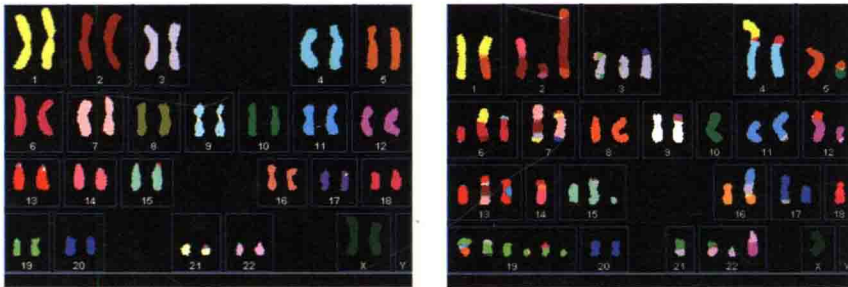
The primordial tumorigenic cell [...] is, according to my hypothesis, a cell that harbours a specific faulty assembly of chromosomes as a consequence of an abnormal event.

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 Geneviève 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.

CONTENTS

CHAPTER 1	RESTARTING DNA REPLICATION BY RECOMBINATION	1
CHAPTER 2	DOUBLE-STRAND BREAK REPAIR PATHWAYS	13
CHAPTER 3	RecA/Rad51 AND THE SEARCH FOR HOMOLOGY	19
CHAPTER 4	PREPARATION OF THE RecA/Rad51 FILAMENT	45
CHAPTER 5	SINGLE-STRAND ANNEALING	73
CHAPTER 6	GENE CONVERSION	89
CHAPTER 7	"IN VIVO BIOCHEMISTRY": RECOMBINATION IN YEAST	127
CHAPTER 8	BREAK-INDUCED REPLICATION	161
CHAPTER 9	SISTER CHROMATID REPAIR	195
CHAPTER 10	GENE TARGETING	215
CHAPTER 11	SITE-SPECIFIC RECOMBINATION	243
CHAPTER 12	CYTOLOGY AND GENETICS OF MEIOSIS	255
CHAPTER 13	MOLECULAR EVENTS DURING MEIOTIC RECOMBINATION	287
CHAPTER 14	HOLLIDAY JUNCTION RESOLVASES AND CROSSING OVER	333
CHAPTER 15	NONHOMOLOGOUS END-JOINING	353
CHAPTER 16	DNA DAMAGE CHECKPOINTS AND GENOME INSTABILITY	367
APPENDIX	EVOLUTION OF MODELS OF HOMOLOGOUS RECOMBINATION (AVAILABLE ONLINE)	
INDEX		383

DETAILED CONTENTS

CHAPTER 1 RESTARTING DNA REPLICATION BY RECOMBINATION

1.1	DNA BREAKS OCCUR FREQUENTLY DURING REPLICATION	1
1.2	LEADING- AND LAGGING-STRAND DNA SYNTHESSES ARE COORDINATED AT THE REPLICATION FORK	3
1.3	REPLICATION FORK STALLING MAY OCCUR IN SEVERAL DIFFERENT WAYS	4
1.4	AN INTRODUCTION TO THE HOLLIDAY JUNCTION	6
1.5	A HOLLIDAY JUNCTION CAN BE RESOLVED BY ENZYMATIC CLEAVAGE	9
1.6	HJ RESOLVASES CAN PROMOTE REPLICATION RESTART BY BREAK-INDUCED REPLICATION	10
	SUMMARY	12
	SUGGESTED READING	12

CHAPTER 2 DOUBLE-STRAND BREAK REPAIR PATHWAYS

2.1	SOME DNA REPAIR OCCURS AT SINGLE-STRANDED GAPS	13
2.2	REPAIR OF DSBs CAN OCCUR IN SEVERAL WAYS	13
	SUMMARY	17
	SUGGESTED READING	17

CHAPTER 3 RecA/Rad51 AND THE SEARCH FOR HOMOLOGY

3.1	RecA AND Rad51 ARE THE KEY STRAND EXCHANGE PROTEINS	19
3.2	FILAMENT ASSEMBLY OF RecA AND Rad51 CAN BE ASSAYED <i>IN VITRO</i>	21

3.3	X-RAY CRYSTALLOGRAPHY HAS REVEALED A GREAT DEAL ABOUT RecA STRUCTURE AND FUNCTION	23
3.4	STRAND EXCHANGE CAN BE STUDIED <i>IN VITRO</i>	25
3.5	STRAND EXCHANGE TAKES PLACE INSIDE THE RecA FILAMENT	28
	A topological approach to studying strand invasion	29
	Single molecule analysis of recombination	30
3.6	<i>IN VITRO</i> ANALYSIS SUGGESTS HOMOLOGY SEARCHING PROCEEDS BY A TETHERED THREE-DIMENSIONAL SEARCH	33
3.7	THE REQUIREMENTS OF HOMOLOGY SEARCHING AND STRAND INVASION CAN BE ASSAYED <i>IN VIVO</i>	34
3.8	BROKEN DNA ENDS BECOME MORE MOBILE	37
3.9	Rad51's PROPERTIES CAN BE ALTERED BY ASSOCIATED PROTEINS	41
3.10	STRAND INVASION CAN APPARENTLY OCCUR WITHOUT Rad51	41
	SUMMARY	43
	SUGGESTED READING	44

CHAPTER 4 PREPARATION OF THE RecA/Rad51 FILAMENT

4.1	DSB ENDS ARE RESECTED IN A 5' TO 3' DIRECTION	45
4.2	RESECTION HAS BEEN WELL STUDIED IN <i>E. COLI</i>	45
4.3	RESECTION IN BUDDING YEAST CAN BE MONITORED <i>IN VIVO</i>	48
4.4	SEVERAL DIFFERENT PROTEIN COMPLEXES ARE INVOLVED IN RESECTION IN BUDDING YEAST	50
4.5	BLM PROTEIN IS IMPORTANT IN TWO EXONUCLEASE COMPLEXES FOR RESECTION IN MAMMALIAN CELLS	54

4.6	RESECTION MUST PASS THROUGH CHROMATIN	55
4.7	PREPARING ssDNA FOR THE LOADING OF RecA/Rad51 REQUIRES ssDNA BINDING PROTEINS	57
4.8	RPA AND Rad51 ASSEMBLY CAN BE ASSAYED <i>IN VIVO</i>	58
4.9	CREATING A RECOMBINASE FILAMENT IN BACTERIA NEEDS THE PARTICIPATION OF SEVERAL MEDIATORS	60
4.10	MEDIATORS IN EUKARYOTES ARE SURPRISINGLY UNRELATED TO THOSE IN BACTERIA	61
4.11	Rad52 AND Rad55–Rad57 ARE THE PRINCIPAL MEDIATORS IN <i>SACCHAROMYCES CEREVISIAE</i>	61
	Rad51 paralogs: Rad55 and Rad57	62
	Additional mediators: the PCSS complex	63
	Rad59, sharing homology with Rad52, is not a mediator but plays an important role	64
4.12	Rad51 FILAMENT ASSEMBLY IN FISSION YEAST INVOLVES EVEN MORE PROTEINS	64
	Rad55/Rad57 versus Swi5/Sfr1	64
4.13	MEDIATORS OF RECOMBINASE FILAMENT ASSEMBLY HAVE BEEN IDENTIFIED IN VERTEBRATE CELLS	67
	Vertebrate Rad51 paralogs	67
	Rad52 and BRCA2	68
4.14	HOMOLOGS OF BRCA2 ARE FOUND IN MANY EUKARYOTES, INCLUDING A YEAST	70
4.15	MANY QUESTIONS REMAIN UNANSWERED	71
	SUMMARY	72
	SUGGESTED READING	72

CHAPTER 5 SINGLE-STRAND ANNEALING

5.1	5' TO 3' RESECTION PROMOTES SINGLE-STRAND ANNEALING	73
5.2	SSA IN BUDDING YEAST CAN BE STUDIED AFTER INDUCING A SITE-SPECIFIC DSB	75
5.3	SSA IS Rad51 INDEPENDENT	78
5.4	SSA DEPENDS ON Rad52's STRAND ANNEALING ACTIVITY	78
5.5	THE REMOVAL OF NONHOMOLOGOUS TAILS IS REQUIRED FOR THE COMPLETION OF SSA	79

5.6	SSA CAN OCCUR BETWEEN MISMATCHED SEQUENCES	81
5.7	THE BEHAVIOR OF DSB ENDS CAN BE EXPLORED USING SSA	83
5.8	SSA CAN BE SEEN IN OTHER ORGANISMS	84
	SUMMARY	87
	SUGGESTED READING	87

CHAPTER 6 GENE CONVERSION

6.1	GENE CONVERSIONS WERE INITIALLY DEFINED FROM ABERRANT SEGREGATION OF ALLELES IN MEIOSIS	89
6.2	ANALOGOUS GENE CONVERSIONS ARISE IN MITOTIC CELLS	90
6.3	MOST GENE CONVERSION ARISES FROM MISMATCH CORRECTION OF HETERODUPLEX DNA	91
6.4	GENE CONVERSIONS CAN BE ACCOMPANIED BY CROSSING OVER	93
6.5	GENE CONVERSION CAN BE ASSAYED IN HAPLOID YEAST	94
6.6	THE MOLECULAR BASIS OF GENE CONVERSION WAS DEDUCED BY RECOMBINING LINEARIZED PLASMID DNA WITH A CHROMOSOME	96
6.7	A QUESTION OF SEMANTICS: CAN THERE BE GENE CONVERSIONS IF THE DONOR AND RECIPIENT CHROMOSOMES ARE IDENTICAL?	100
6.8	HETERODUPLEX CORRECTION OFTEN DEFINES GENE CONVERSION TRACT LENGTHS	100
6.9	WHERE DOES HETERODUPLEX DNA FORM DURING DSB REPAIR?	101
6.10	GENE CONVERSION TRACT LENGTHS HAVE BEEN MEASURED IN BUDDING YEAST	103
6.11	GENE CONVERSIONS ASSOCIATED WITH CROSSING OVER MAY OCCUR BY ALTERNATIVE MECHANISMS	104
6.12	THERE ARE ALTERNATIVE WAYS TO GENERATE CROSSOVERS	105
6.13	RECOMBINATION BETWEEN SEQUENCES OF LIMITED HOMOLOGY LENGTH CONSTRAINS MEASURING hDNA	106
6.14	GENE CONVERSION TRACT LENGTHS ARE VERY DIFFERENT IN MITOTIC AND MEIOTIC YEAST CELLS	109
6.15	A COMPLICATION: HETEROLOGIES INTRODUCE UNCERTAINTY	110

6.16	THERE IS COMPETITION AMONG POSSIBLE GENE CONVERSION DONORS	111	7.7	A NUCLEOSOME PROTECTION ASSAY REVEALS THAT STRAND INVASION ALSO INVOLVES CHROMATIN REMODELING	134
6.17	GENE CONVERSION IS MUTAGENIC: EVIDENCE FOR REDUCED DNA POLYMERASE PROCESSIVITY	112	7.8	THE CAPTURE OF THE SECOND HOMOLOGOUS END DURING GENE CONVERSION CAN ALSO BE STUDIED	138
6.18	GENE CONVERSION FREQUENCIES ARE INFLUENCED BY CHROMOSOMAL POSITION	113	7.9	A SMALL FRACTION OF ECTOPIC GENE CONVERSION EVENTS ARE CROSSOVER ASSOCIATED	139
6.19	GENE CONVERSION AND GENE CONVERSION TRACTS HAVE BEEN DEFINED IN OTHER MODEL ORGANISMS	113	7.10	TWO OTHER HELICASES REGULATE GENE CONVERSION OUTCOMES	140
	<i>S. pombe</i>	113	7.11	DSB BREAK REPAIR IS SURPRISINGLY DIFFERENT FROM GAP REPAIR	141
	<i>Drosophila</i>	116	7.12	STUDY OF MISMATCH CORRECTION DURING STRAND INVASION RAISES QUESTIONS ABOUT HOW <i>MAT</i> SWITCHING OCCURS	146
6.20	HOMOLOGOUS RECOMBINATION CAN BE ANALYZED IN PLANTS	119	7.13	ANOTHER SPECIAL FEATURE OF <i>MAT</i> SWITCHING IS DONOR PREFERENCE	147
6.21	GENE CONVERSIONS HAVE BEEN STUDIED IN MAMMALS	120	7.14	MATING-TYPE SWITCHING IN <i>S. POMBE</i> IS SURPRISINGLY DIFFERENT FROM THAT IN <i>S. CEREVISIAE</i>	148
6.22	CROSSING OVER ACCOMPANYING GENE CONVERSION IS RARE IN MAMMALIAN CELLS	122	7.15	FISSION YEAST <i>MAT1</i> SWITCHING DONOR PREFERENCE INVOLVES CHROMATIN REMODELING	154
6.23	SOME DSB REPAIR EVENTS BEGIN BY HOMOLOGOUS RECOMBINATION BUT TERMINATE WITH A NONHOMOLOGOUS END	123	7.16	A <i>MAT1</i> DSB IS NOT LETHAL IN THE ABSENCE OF THE DONORS	157
SUMMARY		125	7.17	ARE THERE RECOMBINATION ENHANCERS IN OTHER PROGRAMMED RECOMBINATION EVENTS?	159
SUGGESTED READING		125	SUMMARY		159
			SUGGESTED READING		160

CHAPTER 7 "IN VIVO BIOCHEMISTRY": RECOMBINATION IN YEAST

7.1	BUDDING YEAST <i>MAT</i> SWITCHING ALLOWS US TO DESCRIBE THE MOLECULAR EVENTS DURING A GENE CONVERSION EVENT	127
7.2	<i>MAT</i> SWITCHING CAN BE PHYSICALLY MONITORED ON SOUTHERN BLOTS	128
7.3	THE LOADING OF Rad51 ON ssDNA CAN BE VISUALIZED BY CHROMATIN IMMUNOPRECIPITATION	128
7.4	THE ENCOUNTER OF THE Rad51 FILAMENT WITH THE DONOR LOCUS CAN ALSO BE MONITORED BY CHROMATIN IMMUNOPRECIPITATION	130
7.5	A PCR ASSAY CAN BE USED TO DETECT THE BEGINNING OF NEW DNA SYNTHESIS	133
7.6	HEAVY ISOTOPE LABELING CAN BE USED TO SHOW THAT NEW DNA SYNTHESIS IS "CONSERVATIVE"	134

CHAPTER 8 BREAK-INDUCED REPLICATION

8.1	BIR IS IMPORTANT IN THE MATURATION AND REPLICATION OF BACTERIOPHAGE	161
8.2	BIR IS ALSO IMPORTANT IN <i>E. COLI</i>	165
8.3	BIR HAS BEEN WELL DOCUMENTED IN BUDDING YEAST	166
8.4	BIR IS USUALLY Rad51 DEPENDENT	170
8.5	Rad51-DEPENDENT BIR REQUIRES ALL THREE MAJOR DNA POLYMERASES	173
8.6	MANY OTHER GENES AFFECT THE EFFICIENCY OF BIR	174

8.7	REPLICATION DURING BIR IS FAR MORE MUTAGENIC THAN NORMAL REPLICATION	176
8.8	HOW BIR IS FINALLY RESOLVED IS NOT YET KNOWN	177
8.9	THERE IS ALSO A Rad51-INDEPENDENT BIR PATHWAY	179
8.10	HALF-CROSSOVERS ARE AN ALTERNATIVE PATHWAY PRODUCING NONRECIPROCAL TRANSLOCATIONS	181
8.11	BIR IS OBSERVED IN OTHER ORGANISMS	182
	Telomere elongation in <i>Kluyveromyces lactis</i>	182
	BIR in <i>Schizosaccharomyces pombe</i>	183
	<i>Drosophila melanogaster</i> telomere elongation	184
	BIR in <i>Xenopus laevis</i> extracts	184
8.12	BIR-LIKE EVENTS ARE IMPORTANT FOR HUMANS	185
8.13	THERE IS A Rad52-INDEPENDENT HOMOLOGOUS RECOMBINATION PATHWAY	186
8.14	BIR CAN PRODUCE COPY NUMBER VARIATION	189
	Segmental duplications arise in budding yeast	189
	Nonrecurrent SDs in human disease may involve BIR	191
8.15	CNV MAY ARISE FROM MICROHOMOLOGY- MEDIATED BIR	192
8.16	CHROMOTHRIPSIS IS AN UNEXPECTED TYPE OF GENOME INSTABILITY THAT MAY REQUIRE BIR	193
	SUMMARY	194
	SUGGESTED READING	194

CHAPTER 9 SISTER CHROMATID REPAIR

9.1	HOMOLOGOUS RECOMBINATION IS REQUIRED AFTER IONIZING RADIATION	195
9.2	SISTER CHROMATID REPAIR IS PREFERRED OVER RECOMBINATION WITH A HOMOLOG	196
9.3	SCR CAN BE VISUALIZED ON CHROMOSOME- SEPARATING GELS	197
9.4	SOME SISTER CHROMATID REPAIR IS SEEN AS SISTER CHROMATID EXCHANGE	200
9.5	BrdU LABELING IN MAMMALIAN CELLS REVEALS "HARLEQUIN" CHROMOSOMES	202
9.6	SCE CAN ALSO BEEN SEEN BY USING THE CO-FISH TECHNIQUE	204

9.7	SCE CAN BE ASSAYED GENETICALLY BY STUDYING UNEQUAL SCE AND LONG-TRACT GENE CONVERSION	205
9.8	THE GENETIC REQUIREMENTS FOR SPONTANEOUS SCR DIFFER FROM THOSE SEEN IN INTERHOMOLOG REPAIR	207
9.9	SCE CAN BE ANALYZED GENETICALLY IN MAMMALIAN CELLS	208
9.10	RADIO-RESISTANCE IN <i>DEINOCOCCUS</i> <i>RADIODURANS</i> INVOLVES BOTH EFFICIENT DNA REPAIR AND RESISTANCE TO OTHER OXIDATIVE DAMAGE	210
9.11	SISTER CHROMATID REPAIR LEAVES SEVERAL IMPORTANT QUESTIONS UNANSWERED	211
	SUMMARY	213
	SUGGESTED READING	213

CHAPTER 10 GENE TARGETING

10.1	BACTERIAL TRANSFORMATION PROVIDED THE FIRST EVIDENCE OF GENE TARGETING	215
10.2	GENE CORRECTION AND MODIFICATION IN A EUKARYOTE WAS FIRST ACCOMPLISHED IN BUDDING YEAST	215
10.3	DOUBLE-STRAND BREAKS GREATLY IMPROVE GENE TARGETING	217
10.4	GENE TARGETING IS MORE DIFFICULT IN MAMMALS THAN IN YEAST	222
10.5	GENE TARGETING IS IMPROVED BY CREATING A CHROMOSOMAL DSB AT THE TARGET LOCUS	223
	Modifications of an existing site-specific endonuclease	225
	Zinc-finger nucleases	225
	TALE nucleases (TALENs)	225
	CRISPR nucleases	227
10.6	DESIGNER MEGANUCLEASES MAKE IT POSSIBLE TO THINK ABOUT GENETIC SURGERY	228
10.7	CONDITIONAL GENE KNOCKOUTS MAKE IT POSSIBLE TO ANALYZE ESSENTIAL GENES	229
10.8	ENDS-OUT TRANSFORMATION LIKELY HAPPENS THROUGH SEVERAL DIFFERENT PATHWAYS	230
	Hit-and-run transformation	231
	Assimilation of a single strand of transforming DNA	232
	Independent strand invasions	233

10.9	ENDS-IN TARGETING IS MUCH MORE EFFICIENT THAN ENDS-OUT TARGETING	234	12.7	BRANCH MIGRATION CAN LEAD TO AB4:4 SEGREGATION	267
10.10	GENE KNOCKOUTS AND GENE MODIFICATION ARE EFFICIENT IN MODIFIED BACTERIA	236	12.8	GENE CONVERSION AND CROSSING OVER HAVE A STRONG CORRELATION	268
10.11	GENE TARGETING STRATEGIES CAN BE ADAPTED TO OTHER ORGANISMS	238	12.9	MAJOR EVENTS IN MEIOSIS CAN BE OBSERVED CYTOLOGICALLY	269
	Gene modification in <i>Drosophila</i>	239	12.10	SC ASSEMBLY CAN PROCEED IN TWO DISTINCT WAYS	272
	Plants have lagged behind in gene modification	240	12.11	CHROMOSOMES EXHIBIT DYNAMIC MOVEMENTS PRIOR TO ZYGOTENE	272
	SUMMARY	241	12.12	A STRONG BIAS FAVORS INTERHOMOLOG OVER INTERSISTER RECOMBINATION	275
	SUGGESTED READING	241	12.13	SOME MEIOTIC MUTANTS CAN BE ANALYZED BY BYPASSING MEIOSIS I	276

CHAPTER 11 SITE-SPECIFIC RECOMBINATION

11.1	PHASE VARIATION IN <i>SALMONELLA</i> DEPENDS ON A SITE-SPECIFIC RECOMBINASE	243	12.14	RETURN-TO-GROWTH EXPERIMENTS REVEAL A PERIOD OF COMMITMENT TO MEIOTIC LEVELS OF RECOMBINATION	278
11.2	Cre RECOMBINASE RECOMBINES AT A PAIR OF <i>lox</i> SITES	245	12.15	INTERFERENCE REGULATES THE DISTRIBUTION OF CROSSOVERS	279
11.3	Cre-MEDIATED RECOMBINATION CAN BE USED TO CREATE GENOME MODIFICATIONS	246	12.16	HOMEOSTASIS ASSURES THAT SMALL CHROMOSOMES USUALLY GET AT LEAST ONE CROSSOVER DURING MEIOSIS	281
11.4	FLP RECOMBINASE EXCHANGES BETWEEN FRT SITES	249	12.17	THERE ARE SEVERAL MODELS OF INTERFERENCE	283
11.5	Φ C31 INTEGRATION CAN TRANSFER LARGE CHROMOSOMAL SEGMENTS	250		SUMMARY	285
	SUMMARY	254		SUGGESTED READING	285
	SUGGESTED READING	254			

CHAPTER 12 CYTOLOGY AND GENETICS OF MEIOSIS

12.1	RECOMBINATION IS REQUIRED TO GENERATE DIVERSITY BUT ALSO TO ENSURE CHROMOSOME SEGREGATION	255	13.1	SPO11 CREATES DSBs TO INITIATE MEIOTIC RECOMBINATION	287
12.2	EARLY MEIOSIS IN <i>DROSOPHILA</i> STUDIES SHOWED THAT RECOMBINATION OCCURS AFTER CHROMOSOME REPLICATION	257	13.2	DSB FORMATION IS ASSOCIATED WITH REPLICATION AND CHROMATIN MODIFICATIONS	288
12.3	IN FUNGI, ALL FOUR PRODUCTS OF A SINGLE MEIOSIS CAN BE RECOVERED	258	13.3	MEIOTIC HOT SPOTS CAN BE IDENTIFIED IN MANY DIFFERENT ORGANISMS	290
12.4	GENE CONVERSIONS ARISE FREQUENTLY IN MEIOSIS	261		Fission yeast	295
12.5	POST-MEIOTIC SEGREGATION IS SEEN IN THE ABSENCE OF MISMATCH REPAIR	263		Mammals	295
12.6	MEIOTIC SEGREGATION PATTERNS REVEAL HOW RECOMBINATION IS INITIATED	265	13.4	MEIOTIC HOT SPOTS IN MAMMALS CORRELATE STRONGLY WITH PRDM9 HISTONE METHYLTRANSFERASE	296
			13.5	HOMOLOGOUS CHROMOSOME PAIRING OFTEN REQUIRES Spo11-INDUCED RECOMBINATION	299

CHAPTER 13 MOLECULAR EVENTS DURING MEIOTIC RECOMBINATION

13.6	RECOMBINATION CANNOT BEGIN UNTIL Spo11 IS RELEASED FROM THE DSB ENDS	299	13.19	GLOBAL ANALYSIS OF MEIOTIC EVENTS HAS BEEN EXTENDED TO METAZOANS AND PLANTS	327
13.7	IN MEIOSIS, IN MANY ORGANISMS, Rad51 IS NOT THE ONLY RecA-LIKE STRAND EXCHANGE PROTEIN	300		<i>Drosophila</i>	327
13.8	Dmc1 WORKS WITH SEVERAL MEIOSIS-SPECIFIC AUXILIARY FACTORS	301		<i>Arabidopsis</i>	327
13.9	Rad51 PLAYS ONLY A SUPPORTING ROLE IN YEAST MEIOSIS	302		Mouse	327
13.10	MOLECULAR INTERMEDIATES OF MEIOTIC RECOMBINATION ARE WELL-STUDIED IN BUDDING YEAST	304		Humans	328
	Crossover products	306	13.20	MEIOTIC RECOMBINATION CAN BE INDUCED BY MEGANUCLEASE-INDUCED DSBs	328
	DNA end resection in meiosis is less extensive than in mitotic cells	307		HO endonuclease	328
	Binding of Dmc1 and Rad51 to DSB ends	307		VDE	330
13.11	TRANSIENT STRAND INVASION INTERMEDIATES CAN BE IDENTIFIED BY 2-D GEL ELECTROPHORESIS	308		I-SceI cleavage in <i>S. pombe</i> meiosis	330
	Single-end invasion	308		<i>Mos1</i> transposon	330
	dHJ formation	309		Gamma irradiation	331
	Initiation of new DNA synthesis	311		SUMMARY	331
	HJ formation in <i>S. pombe</i>	312		SUGGESTED READING	331
13.12	TO COMPLETE GENE CONVERSION THE SECOND DSB END MUST BE CAPTURED	312	CHAPTER 14 HOLLIDAY JUNCTION RESOLVASES AND CROSSING OVER		
13.13	AXIAL ELEMENT COMPONENTS ARE IMPORTANT IN THE CONTROL OF THE INTERHOMOLOG BIAS	315	14.1	HOLLIDAY JUNCTIONS CAN ADOPT ALTERNATIVE CONFIGURATIONS	333
13.14	ZMM PROTEINS PLAY KEY ROLES IN REGULATING CROSSOVERS AND IMPLEMENTING INTERFERENCE	317	14.2	CANONICAL HJ RESOLVASES MAKE SYMMETRICAL CUTS THAT CAN BE RE-LIGATED	334
	Msh4–Msh5	317	14.3	NONCANONICAL RESOLVASES MAY ACT ON NICKED HJs	338
	Mlh1/Mlh3	318	14.4	THERE ARE AT LEAST FOUR HJ RESOLVASES IN EUKARYOTES	338
	Mer3	319		Mus81 complex	338
	Zip1/Zip2/Zip3/Zip4/Spo16	320		Yen1/Gen1	339
13.15	ZMM PROTEINS ARE EQUALLY IMPORTANT IN MEIOSIS OF METAZOANS AND PLANTS	321		Slx1–Slx4	340
13.16	THE CROSSOVER/NONCROSSOVER DECISION APPEARS TO BE MADE EARLY IN DSB REPAIR	321		Exo1–Mlh1–Mlh3	340
13.17	AT LEAST ONE MORE CROSSOVER SYSTEM EXISTS, IN ADDITION TO THAT CONTROLLED BY ZMM PROTEINS	322	14.5	DIFFERENT HJ RESOLVASES PLAY DIFFERENT ROLES IN DIFFERENT ORGANISMS	340
13.18	DISTRIBUTION OF CROSSOVERS AND NONCROSSOVERS CAN BE ANALYZED GENOMEWIDE IN BUDDING YEAST	324		Budding yeast mitotic cells	341
				Budding yeast meiosis	343
				Fission yeast	344
				<i>Drosophila</i>	345
				<i>Caenorhabditis</i>	346
				Mammals	346
			14.6	BRANCH MIGRATION ENZYMES CAN INFLUENCE HJ CLEAVAGE	347
			14.7	MISMATCH REPAIR ALSO INFLUENCES CROSSOVER REGULATION	348
				SUMMARY	350
				SUGGESTED READING	351

CHAPTER 15 NONHOMOLOGOUS
END-JOINING

15.1	“CLASSICAL” NHEJ IS ESSENTIAL FOR THE MAMMALIAN IMMUNE SYSTEM	353
15.2	VDJ JOININGS OFTEN EXHIBIT ADDITIONAL MODIFICATIONS AT THE JUNCTION	356
15.3	NHEJ CONTRIBUTES TO DSB REPAIR IN YEAST	357
15.4	NHEJ FACILITATES CAPTURE OF DNA FRAGMENTS AT DSBs	360
15.5	END-JOINING MAY ALSO OCCUR BY ALTERNATIVE NHEJ	361
15.6	THE 53BP1 PROTEIN PLAYS MULTIPLE ROLES IN END-JOINING	363
15.7	GENE AMPLIFICATION CAN OCCUR VIA NHEJ AND BFB CYCLES	364
	SUMMARY	365
	SUGGESTED READING	366

CHAPTER 16 DNA DAMAGE
CHECKPOINTS AND GENOME
INSTABILITY

16.1	THE DNA DAMAGE CHECKPOINT PROVIDES A CELL CYCLE DELAY TO ALLOW DNA REPAIR	367
16.2	PI3 KINASE-LIKE KINASES ARE AT THE APEX OF DNA DAMAGE SIGNALING	368
16.3	DIFFERENT MECHANISMS ACTIVATE ATM AND ATR IN RESPONSE TO A DSB	369

16.4	ATM AND ATR INITIATE A PROTEIN KINASE CASCADE	372
16.5	DSB-INDUCED CELL CYCLE ARREST IN MAMMALS OCCURS BEFORE S PHASE AND MITOSIS	373
16.6	DSB-INDUCED CELL CYCLE ARREST IN BUDDING YEAST OCCURS PRIMARILY BEFORE ANAPHASE	374
16.7	γ -H2AX IS IMPORTANT FOR SISTER CHROMATID REPAIR IN MAMMALS	375
16.8	THE DNA DAMAGE CHECKPOINT MODULATES DSB REPAIR IN MANY WAYS	376
16.9	FAILURES OF THE DNA DAMAGE RESPONSE CONTRIBUTE TO GENOME INSTABILITY	377
16.10	CANCER CHEMOTHERAPIES EXPLOIT TARGETS IN MULTIPLE DNA REPAIR PATHWAYS	379
16.11	HOMOLOGOUS RECOMBINATION TURNS UP IN STEM CELL REPROGRAMMING	380
	SUMMARY	380
	SUGGESTED READING	381

APPENDIX: EVOLUTION OF
MODELS OF HOMOLOGOUS
RECOMBINATION (AVAILABLE ONLINE)

INDEX 383