

## Molecular Biosafety

Margy S. Lambert

University of Wisconsin—Madison, Madison, Wisconsin

The molecular biology and biotechnology fields are growing by leaps and bounds. Molecular Biosafety aims to shed light on how these cutting-edge techniques impact safety. Please e-mail your insights and questions to Margy Lambert at [mlambert@fpm.wisc.edu](mailto:mlambert@fpm.wisc.edu) or Co-Editor Barbara Johnson at [barbara\\_johnson@verizon.net](mailto:barbara_johnson@verizon.net) or Co-Editor Karen B. Byers at [karen\\_byers@dfci.harvard.edu](mailto:karen_byers@dfci.harvard.edu).

### A Primer on Recombination and What It Means to Biological Safety

Recombination is a simple concept with complex mechanisms and far-reaching consequences. A basic description of genetic recombination is that it is a process where segments of nucleic acid molecules interact and are recombined or re-assorted or exchanged (Snustad & Simmons, 2006). This article focuses on the types of recombination involving re-attachment or recombination of nucleic acid strands that have become broken due to natural cellular processes, recombinant techniques, or induction by environmental triggers. Recombination can serve many purposes (Table 1), but a key overall function is to act as an agent of change—for good and for bad.

There are many different ways to categorize the widely divergent types of recombination. One categorization scheme is given in Table 2. In prokaryotes such as

bacteria, recombination occurs in the bacterial chromosome as well as in RNA and extrachromosomal DNA such as plasmids and viral DNA (e.g., bacteriophages). Eukaryotes demonstrate recombination in chromosomes as well as in RNA and in extrachromosomal DNA such as mitochondrial DNA. With recombinant expression systems, plasmids are commonly introduced into mammalian cell lines representing prokaryotic nucleic acids in a eukaryotic environment.

Recombinational mutations can lead to chromosomal rearrangements such as translocations and deletions. In meiosis, such mutations in reproductive tissues can be observed as harmless variations or sometimes as birth defects in offspring, while recombinational mutations in somatic tissues have the possible end result of cancer.

The mechanisms of recombination systems are very complex. Homologous recombination requires stretches of homologous sequence in the substrates for recombination events to occur. Nonhomologous recombination is of two types—illegitimate and site-specific—with illegitimate requiring no substrate homology while site-specific recombination needs short regions of homologies as binding sites for the necessary recombination complex. There is often a two-way exchange (reciprocal recombination), but there can also be a one-way transfer such as transposition “jumping genes” and one-way transfers

**Table 1**  
Major Purposes of Recombination

Purpose	Example/Description
Gene Regulation	Rearranging regulatory elements (e.g., promoters, enhancers) to alter regulation of genes
DNA repair	Recombination provides necessary components for repair mechanisms (e.g., insertion of repaired DNA sequence into the sequence flanking the region repaired)
Diversity	Example 1: reassortment of chromosomes in meiosis
	Example 2: reassortment of genes in some viruses such as influenza
	Example 3: programmed site-specific recombination in mammalian immune system (immunoglobulins and T-cell receptors)
Evolution	Source of mutations with selection for or against in the evolutionary process
Chromosomal Segregation in Meiosis	Needed for appropriate segregation of homologous chromosomes in meiosis

Notes: Some of the functions such as diversity and evolution are related, but are listed separately in this table. Adapted from “Development of a Human Recombinational Mutation Assay and a Mechanistic Model for the Chromosomal Rearrangements in Cancer,” Doctoral dissertation of Margy S. Lambert, Table 4.

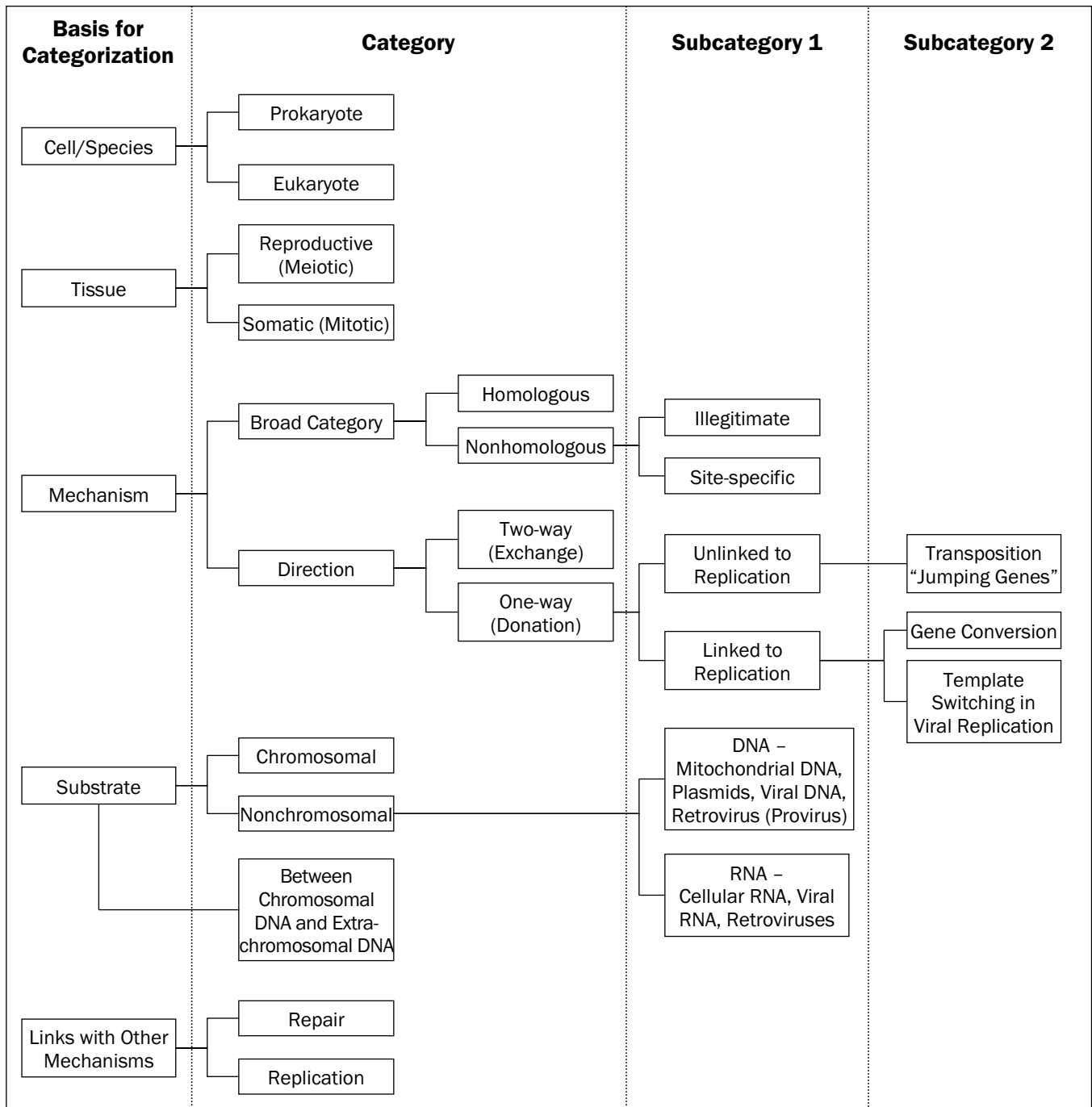
linked to replication (e.g., gene conversion). In the case of viruses, the recombination machinery is sometimes supplied by the host.

Genomic DNA is only one substrate that undergoes recombination. RNA and extrachromosomal DNA are also participants in recombination events. Extrachromosomal DNA, such as mitochondrial DNA, plasmids, DNA viral genomes, and the DNA stage of retroviruses (provirus), can act as substrates in recombination systems.

Cellular RNAs, RNA viral genomes, and the RNA stage of retroviruses can all be substrates for recombination too. Recombination can also occur between chromosomal and extrachromosomal DNA; examples of this are the integration of viral DNA and of transposons into chromosomes. Both of these types of events can potentially result in insertional mutagenesis.

Recombination in chromosomal DNA generally has a more long-term impact than recombination in extrachro-

**Table 2**  
Categories of Recombination



mosomal DNA or in RNA. There is turnover of cellular extrachromosomal DNA and RNA, while chromosomal DNA in eukaryotes is passed on from one generation of cells to the next in somatic tissues and from one generation of organisms to the next in reproductive tissues.

Replication, recombination, and repair have often been studied as separate molecular events, but in reality these pathways have been found to be closely linked (Hanawalt, 2007). Homologous and nonhomologous recombination mechanisms are essential components in the major DNA repair pathways: Damaged DNA must be excised and repaired DNA must be integrated back into the DNA molecule. The close link between recombination and replication is evidenced as gene conversion events in genomic DNA. Gene conversion events that result in loss of heterozygosity at tumor suppressor genes such as p53 are important steps in some types of cancer. The most common category of recombination events in viral RNA genomes (template switching) occurs in conjunction with replication of the viral genome (Figlerowicz & Bibillo, 2000).

From a biosafety perspective, recombination plays a major role in risk assessment of pathogens mainly because it represents a key route to change in nucleic acids. Recombination produces diversity that evolutionary forces can then act upon. Many of the changes may be detrimental, but a few will provide a selective advantage for survival in a changing environment.

Recombination can result in altered genes or regulation of genes that increases hazards in pathogens, including changes that increase virulence or pathogenicity, broadens host or tissue ranges, allows generation of a replication-competent virus from a replication-deficient virus, or causes insertional mutagenesis. Insertional mutagenesis can sometimes lead to cancer or to reproductive effects. A key question is how to best incorporate evaluation of the potential risks of recombination into assessments of research involving pathogens.

Ongoing development of lentiviral vector systems is an excellent example of adjusting methodology to reduce risks following evaluation of recombination potential. The current generation of lentiviral vectors has a

number of safety features engineered into the systems. A primary safety characteristic that greatly reduces the possibility of replication-competent virus arising through recombination is the use of a multiplasmid transfection method with the necessary replication genes on separate plasmids. This method ensures that several rare recombination events would need to occur in order for a replication-competent virus to be generated.

Information on recombination for specific sets of circumstances is often difficult to find. For example, recombination frequency estimates for experimental conditions of interest are sometimes limited or unavailable. On the other hand, the body of knowledge for recombinational systems is rapidly growing. Evaluating recombination potential as part of risk assessments is not an exact science or an easy task. But considering the potential consequences of increasing pathogen hazards due to recombination events enables more thorough risk assessments. Such an evaluation, as exemplified in development of safer lentiviral vector systems, can also lead to insights into how to best minimize any potential recombination risks that may exist.

#### Author's Note

See upcoming Molecular Biosafety columns in this journal for further discussion on evaluating potential recombination risks for various types of pathogens.

#### References

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