

Molecular Biosafety

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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@mcw.edu or Co-Editor Barbara Johnson at barbara_johnson@verizon.net or Co-Editor Karen B. Byers at karen_byers@dfci.harvard.edu.

Recombination and Horizontal Gene Transfer: Routes to Increased Bacterial Pathogenicity

A previous Molecular Biosafety column (Lambert, 2008) laid out the basic premise for why the potential for genetic recombination should be considered in risk assessments of pathogens: because recombination represents a key route to change in nucleic acids. The current article continues this series with a focus on recombination in bacterial pathogens.

Bacterial Recombination

Introduction of new genetic material/altered sequences into bacteria can be through several mechanisms including:

- Mutations: Some mutational mechanisms involve recombination, resulting in rearrangements such as deletions, duplications, or insertions.
- DNA Repair: Most DNA repair mechanisms involve recombination at some level. For bacteria, a main route of repair is homologous recombinational repair which has recombination as its centerpiece.
- Horizontal Gene Transfer (HGT): The introduction of extrachromosomal mobile units plays a central role in bacteria gaining genetic material. HGT allows bacteria to respond quickly to changing environments and to exploit new ecological niches (Sobecky & Coombs, 2009). HGT is largely responsible for the spread of fitness-enhancing traits, including antibiotic resistance and virulence factors (Averhoff, 2009).

In bacteria, genetic material is often introduced via HGT with mobile genetic elements such as plasmids (conjugation) or bacteriophages (transduction) and through the uptake of naked DNA that is integrated into the bacterial chromosome (natural transformation) (Kelly et al., 2009). Though recombination is not involved in HGT itself, DNA that has been introduced via HGT can then be recombined with other mobile genetic elements or with the bacterial chromosome. The bacterial environment is so dynamic that new advantageous genetic ma-

terial can be maintained on mobile genetic elements indefinitely through selective pressure. However, genetic material that benefits the bacterial host is also maintained through integration into the bacterial chromosome (through recombination mechanisms including transposition “jumping genes”). Integration enables vertical gene transfer (VGT) when the bacteria replicates.

Thus, recombination plays a major role in the introduction of genetic material to bacteria, which is counterbalanced by HGT. Likewise, recombination plays a major role in the maintenance of gene expression through integration into the bacterial genome, but this role for recombination is also counterbalanced by selective pressure that results in the maintenance of new genetic material that is beneficial to that specific bacteria in that particular environment.

Risk Assessment Considerations for Recombination in Bacterial Pathogens

Risk assessments of bacterial pathogens need to take into account whether introduced changes can result in increased virulence, increased pathogenicity, broadened host or tissue ranges, increased routes of transmission, decreases in infectious dose, increased survival in the environment, or increased resistance to antibiotics.

HGT is often facilitated by genomic islands (GIs), discrete DNA segments which can be mobile. Many GIs can integrate into the host chromosome, be readily excised, and transfer to a new host by transformation, conjugation, or transduction. GIs involved in processes affecting pathogenicity, such as rapid dissemination of genes coding for antibiotic resistance genes and for virulence genes, are termed pathogenicity islands (Juhás et al., 2009). The rapid evolution of pathogenicity islands involves both HGT and recombination mechanisms.

Type IV secretion systems are conjugation-related genes that are very versatile. They can be involved in protein secretion and in the export and import of novel DNA sequences that are then integrated into the bacterial genome (natural transformation). Thus, type IV secretion systems are tools for HGT and for increasing virulence (Juhás et al., 2008).

Biofilms are microbial communities that are matrix-embedded and consist of interacting microorganisms. The organisms within a biofilm communicate by gene transfer and by secretion of signaling molecules. Gene transfer mechanisms in biofilms often involve HGT, but may also include recombination mechanisms. Biofilm formation in pathogens often results in increased virulence and decreased susceptibility to antimicrobial

agents (Marsh, 2005).

One review article (Ambur et al., 2009) evaluates genome dynamics in a number of major bacterial pathogens with a focus on key mechanisms (DNA repair, recombination, and HGT) that enable maintenance of bacterial genome integrity. DNA repair mechanisms (with recombination generally having a fundamental role in the mechanism) contribute to the ability of bacteria to colonize, transmit, and survive inside the host. While the absence of DNA repair activities may be beneficial and allow adaptation during some stages of a pathogen's life cycle, intact repair systems are essential for long-term colonization. The Ambur review article concludes that these divergent needs regarding DNA repair can be met through bacterial pathogens having mechanisms such as HGT for speedily reacquiring genes encoding DNA repair functions.

The relative importance of recombination as the mechanism of change is species-specific and sometimes even strain-specific and is also dependent on the adaptive response in the host of that pathogen. *Streptococcus pyogenes*, for example, demonstrates high levels of recombination and a narrow habitat (Bessen, 2009) while *Streptococcus pneumoniae* has a high level of HGT by natural transformation (Johnsberg & Havarstein, 2009). *Mycobacterium tuberculosis* shows significant genetic diversity generated by recombination mechanisms, but unlike many bacterial pathogens, gene exchange is rare resulting in distinct clonal lineages in *M. tuberculosis* (Nicol & Wilkinson, 2008). Population studies indicate that recombinational mechanisms play a large role in speedy acquisition of antibiotic resistance in commensal species of *Neisseria meningitidis* (Sáez-Nieto & Vazquez, 1997). Putative virulence factors of *Helicobacter pylori* are numerous and are often acquired through recombination events involving pathogenicity islands (Figura, 1997). In *Staphylococcus aureus*, antibiotic resistance genes are often exchanged between organisms via HGT, but chromosomal mutation (which can be via a recombination mechanism) is the catalyst for novel resistance genes (Jensen & Lyon, 2009).

In summary, both HGT and recombination are important mechanisms in acquisition of new traits in bacterial pathogens, and both selective pressures and recombination are important routes to maintain those traits beneficial to that organism in that environment. HGT and recombination mechanisms, however, are often not separable events, but instead are complementing mechanisms that allow bacteria, including pathogenic bacteria, to quickly adapt to changing environments (Sriramulu, 2008).

Doing risk assessments that take into account the various mechanisms that can result in increased bacterial pathogenicity is very complex. It is clear, however, that getting as complete a picture as possible for specific pathogens enables a more thorough risk assess-

ment and allows tailoring of precautions based on the specific risks posed by that particular pathogen.

Author's Note

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

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