

and treated with an additional three months of antibiotic therapy. All symptoms resolved; he returned to work in September 1988, and his antibody titer dropped to 80, indicating a resolved previous infection. However, in February 1989, his wife was admitted to the hospital with fevers, chills, headache, and myalgias. *Brucella* was isolated from her blood culture and the CDC reference laboratory confirmed that her blood culture isolate was identical to the outbreak strain. Person-to-person transmission does not commonly occur in *Brucella*; however, it was suspected in this case. The wife had not visited the microbiology laboratory in years and had no other risk factors for *Brucella* infection. Sexual relations with the infected husband had occurred infrequently during his acute illness June through September, but resumed in October 1988, before his antibiotic treatment for brucellosis was completed. No culture evidence for sexual transmission was obtained from either spouse, but the author advises that it would be prudent to abstain from unprotected intercourse during treatment for *Brucella*.

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## 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).

### Safety Advance: Transposon Gene Delivery Systems

What do the terms “piggyBac,” “*Sleeping Beauty*,” and “*Frog Prince*,” have to do with biological safety? These transposons as well as others, such as *Tol2*, *Mos1*, and *Himar1*, are being used in the development of nonviral DNA/gene delivery systems. The relevance to safety is that nonviral delivery methods have the primary advantage over viral vectors of being noninfectious.

Transposons, or transposable elements, are DNA elements that can move or “transpose” from one location in a DNA molecule to another location, either on the same or a different DNA molecule. The phrase “jumping genes,” coined from Nobel Prize winning scientist Barbara McClintock’s research with maize, may be a more

recognizable expression. Transposases are the enzymes that catalyze the DNA movement. The development of gene delivery systems, commonly referred to as vectors, is often driven by the goal of creating safer and more effective human gene therapy options.

Viruses have the capacity to enter host cells and direct host machinery to produce the components necessary for infectious viral particles (Knipe et al., 2007). Some viruses, such as retrovirus, lentivirus, and adeno-associated virus, can integrate into the host genome with the potential result of chronic or latent infections. Integration can also result in insertional mutagenesis if the viral DNA integrates near a gene such as an oncogene.

Use of replication-deficient viral vectors greatly minimizes the chance that infectious progeny viruses will be generated. The possibility of infectivity is not eliminated; however, because replication-competent viruses can arise through a recombination mechanism. Viral vectors’ major advantage over nonviral methods is the ability to achieve stable expression. In particular, retroviral and lentiviral vectors can integrate into the host genome to achieve long-term expression of the gene of interest. Another potential advantage of viral vectors is the ability to target specific tissues. Tissue tropism is exhibited because viral entry is limited by the type of receptors displayed on

the cell membrane surface, which varies by tissue type.

Disadvantages of viral vectors include the potential for pathogenicity/infectivity, induction of immune inflammatory responses, insertional mutagenesis, and frequently the need to use a higher level of precautions. The risks of replication-deficient viruses gaining the genes necessary to become replication-competent or of retroviruses/lentiviruses integrating near a crucial gene such as an oncogene and activating its expression are slight. However, these risks often necessitate the use of biosafety level 2 (BSL-2) precautions and containment.

The most common nonviral delivery route is via plasmids. Plasmids are extrachromosomal DNA rings that replicate independently of genomic DNA and provide a widely-used versatile tool for manipulating and moving genes from cell to cell. Plasmid vectors have the advantages of simplicity, safety (noninfectious components), and lack of immune activation. BSL-1 precautions and containment are appropriate for use of the vast majority of plasmid vectors. Advances have been made in nonviral delivery methods, but until recently, the major drawback of these systems has been the inability to achieve persistent expression due to loss and degradation of plasmid DNA in living tissues.

A recent advance has been the use of plasmids that include transposons and transposases. Transposon systems overcome the main drawback of simple plasmid delivery systems since the transposases integrate the genes of interest into the host chromosomes resulting in stable expression (Balciunas et al., 2006; Miskey et al., 2003; Wu et al., 2006). Transposase activity can then be turned off to stop movement of DNA. Transposon vectors offer a major step forward in safety because of the elimination of the infectious component of viral vectors. However, insertional mutagenesis remains a potential drawback of transposon delivery systems.

Transposases such as *Sleeping Beauty* have the advantage of near-random integration sites as compared to retroviral/lentiviral vectors that tend to integrate into actively-transcribed regions of the genome. The ease of manipulation of transposon vectors allows for modifications such as coupling of transposases to DNA-binding domains to achieve targeted integration (Kaminski et al., 2002). The use of such chimeric transposases could further improve the safety of transposon vectors by targeting integration to "safe" regions of the genome not associated with oncogenesis.

*Sleeping Beauty* (a synthetic transposon derived from fish) was constructed and demonstrated to be capable of transposing DNA from a plasmid to a human chromosome 10 years ago (Ivics et al., 1997). Since then, a number of transposon systems have been developed and used successfully to obtain stable gene expression in mammalian cells. Transposon vectors have been used effectively in primary and established mammalian cells from various lineages (Huang et al., 2006) as well as in animal models

of gene therapy (Liu et al., 2006). Recently, a new strategy has been devised that uses the *piggyBac* transposon (derived from a moth) for production of transgenic animals (Shinohara et al., 2007).

There are still many unknowns in the development of transposon vectors, including remaining questions on technical as well as safety issues. The use of such vectors, however, offers a great opportunity to maximize the advantages and minimize the drawbacks of existing delivery systems. Transposon delivery systems combine the ability to achieve stable gene expression with the use of safer noninfectious components. In addition, the incorporation of targeted integration mechanisms into transposon systems could significantly decrease the insertional mutagenesis risk. The potential safety benefits alone merit consideration of this method.

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