

# Genetic Development in Spirochetes

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Diverse and ubiquitous, the morphologically captivating spirochetes include the causative agents of various important diseases, *i.e.* syphilis, periodontitis, Lyme disease, Leptospirosis and swine dysentery. The advancement of our knowledge in regard to the biology of this bacterial family has been hampered by the absence of suitable genetic tools. This limitation has been of special significance in the understanding of the mechanism of disease, because the few pathogenic spirochetes that can be cultured *in vitro* are also particularly intractable genetically. The advent of the new millennium is witnessing a revolution of the genetic methodologies available to study spirochetes. The following collection of seven mini-reviews present relevant milestones of this process.

Besides genetic engineering, the manipulation of DNA needed in investigations of gene regulation and protein function requires the ability to transfer DNA to the desired bacterial recipient. Tilly and coworkers exhaustively discuss the methods available for DNA exchange in spirochetes, and their successful application to gene inactivation. Obviously, it is necessary to be able to discriminate those spirochetes carrying the recombinant DNA. Addressing this issue, Hardham and Rosey comprehensively cover in their mini-review, the selective and screening markers proven to be useful in spirochetes, as well as suggest potential alternative antibiotic resistances to be tested.

Although, the transient presence of exogenous DNA fragments has been sufficient to attain homologous recombination and to carry out studies of gene expression, the efficiency, convenience and versatility of these systems is limited when compared with that provided by stable vectors. A remarkable effort has been carried out by a number of laboratories towards obtaining stable episomes capable of shuttling DNA between genetically amenable bacteria and spirochetes. Saint Girons *et al.* describe the recent advances in this area for *Treponema denticola*, *Leptospira biflexa* and *Borrelia burgdorferi*.

The strategies used to obtain stable maintenance of exogenous DNA have been based on broad host range plasmids or on the random cloning of endogenous sequences containing a native replicon from the spirochetal genome. In addition, an innovative approach guided by genome analysis to locate spirochetal origins of replication is extensively elaborated in the mini-review by García-Lara *et al.* Besides its application to the development of shuttle vectors, this methodology has provided the first origins of replication from the unusual linear chromosome and linear plasmids of *Borrelia burgdorferi*. It has also unveiled a

collection of candidate genes to be involved in replication and/or partitioning.

The contribution of genomics to spirochetal research has been certainly catalyzed by the sequencing of the complete genome of *Treponema pallidum* and *Borrelia burgdorferi*. Similarly, the work on outer membrane proteins of *Borrelia* or the treponemes has evolved at a more rapid pace than that of other genres, and it has patterned, for instance, much of the investigations in *Leptospira*. The resulting cross-talk generated by the findings obtained from various spirochetal species, based both, on predictive genomics and experimental results, has rendered exciting observations. For example, the definition of a lipobox for spirochetal lipoproteins, presented by Zuerner and coauthors in their mini-review. They also describe various aspects of leptospiral research that lead the pack of the spirochetes, such as genetic complementation analysis and on studies pertaining to repetitive DNA sequences in the genome.

A complete genetic system for a bacterial species needs to consider the technology required to study the differential gene expression that will take place as the microorganism encounters different environments. In the case of bacterial pathogens, this would include their corresponding hosts. Seshu and Skare provide a thorough presentation of the methodologies and findings on *in vivo* gene expression as it relates to *Borrelia burgdorferi*.

The current knowledge on transcriptional regulators in spirochetes and on the DNA elements that mediate their action is reviewed by Indest *et al.* Amongst the various mechanisms of gene regulation addressed by these authors, they discuss one of the spotlights of current microbiological research, regulation by quorum sensing. The outcome of studies on differential gene expression impacts the basic understanding of the disease process, the search for therapeutic targets, and the design of biotechnological tools.

As the methodologies presented in this collection of articles further develop and the validity of other proposed alternatives is assessed, additional contributions of unquestionable value such as DNA microarray technology will assist in unraveling the mysteries of the biology of spirochetes.

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