Preface

The term "megaplasmid" was first used by Rosenberg and coworkers in 1981 as a designation for plasmids "...with a molecular weight clearly greater than 300×10^6" (Rosenberg et al. 1981). Using gentle lysis techniques to minimize shearing of DNA molecules, these authors prepared lysates of *Sinorhizobium meliloti* cells and examined their plasmid content by means of agarose gel electrophoresis. They found a high-molecular weight species in several different strains of *S. meliloti* and showed that it carried determinants for both symbiosis and nitrogen fixation. As was customary at the time, the size of the DNA element was given as apparent molecular weight (100×10^6 is equivalent to about 150 kb). The high-molecular weight plasmid discovered in *S. meliloti* by Rosenberg and coworkers has been intensively studied in the past two decades, especially the species present in *S. meliloti* strain 1021, which is called pSymA.

The new term for "very large plasmids" was arbitrary in more than one respect. Clearly Rosenberg and his coauthors coined the term simply to indicate plasmids in a size range that had hitherto not been detected. There was no particular reason for the threshold of 300×10^6 (~ 450 kb) and no grounds to assume that these DNA species had biological properties distinguishing them from smaller plasmids. Inherent in the term "megaplasmid" were the tacit assumptions that bacteria have only a single chromosome, i.e. a replicon carrying genetic information that is essential for the cell under all growth conditions, and that, in bacteria harboring multiple replicons, the chromosome is always the largest replicon. Hence, in the original article cited above, the question of essentiality was not raised.

Since the discovery of giant plasmids in *S. meliloti,* our knowledge of bacterial genomes has progressed remarkably. On the one hand, many new plasmids in the size range >100 kb have been discovered in various bacteria and archaea and some have been shown to encode capabilities of immense ecological and industrial significance. More importantly, the advent of whole genome sequencing has led to fundamental changes in our thinking. It is now clear that diverse bacteria, such as *Rhodobacter sphaeroides* 2.4.1, *Brucella suis* and *Burkholderia pseudomallei* to name a few examples, possess more than one chromosome (Bentley and Parkhill 2004). This necessarily casts doubts on the classification of replicons as chromosomes or megaplasmids based on size alone. The case of *S. meliloti*, host of the prototypical megaplasmids, is interesting in this context. Curing experiments established

that the 1.3 Mb replicon pSymA is in fact nonessential and thus is a true megaplasmid. The status of the 1.6 Mb replicon pSymB was for long a matter of debate. Most but not all of pSymB can be deleted without significantly affecting viability. pSymB carries singular copies of a few essential housekeeping genes – a gene for $tRNA^{Arg}_{CCG}$ and the *minCDE* genes – making it essential for the growth of *S. meliloti* (Wong et al. 2002). This example illustrates that it is a very fine line separating chromosomes and megaplasmids, as a single recombinative event can result in the transfer of an essential chromosomal gene to a plasmid. Indeed, it has been proposed that secondary chromosomes originated from megaplasmids via the acquisition of essential housekeeping genes. Many secondary chromosomes have plasmid-type replication origins. Furthermore, analysis of the ParA partitioning proteins reveals that the sequences of ParA proteins deduced for secondary chromosomes cluster with plasmid ParA sequences, suggesting that they are more closely related (MacLellan et al. 2004).

From the above discussion it should be clear that the class of extrachromosomal elements that have been designated as megaplasmids has no sharp boundaries, but rather is an arbitrary segment or slice of the spectrum of microbial replicons. What, then, is the justification for publishing a collection of articles devoted to "megaplasmid biology"?

The giant replicons surveyed in this book contribute to the biology of their hosts in a special way that sets them apart from smaller plasmids. They provide complex biochemical pathways or whole repertoires of gene products that in some cases enable the organism to prevail in special niches, such as H_2-rich soil microcosms or the hostile environment of a mammalian host. Paradoxically, many of the megaplasmids described in the following chapters are nonessential, but carry genetic determinants that define the unique biology of the organisms they inhabit.

For purely pragmatic reasons, a lower threshold of 100 kb was adopted for this selection of megaplasmids. Notwithstanding this limitation it was not possible to include all of the interesting and well-studied megaplasmids in this book. The reader will find that the scope of the various chapters is not uniform: Some chapters survey a lifetime of work, while others present a story that is just beginning to unfold. In our opinion they are all fascinating. To all the authors who in spite of their numerous other duties found time to review the work in their field in a novel vein, we express our gratitude. We are grateful to Springer and especially to Jutta Lindenborn of the editorial staff for her support and counsel.

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