Mycoplasma and *Spiroplasma*

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Glossary

adhesin Bacterial protein that is involved in the adhesion to the eukaryotic host cell.
firmicutes A monophyletic group of Gram-positive bacteria whose genomes possess low GC content. The mollicutes are a subgroup of the firmicutes.
phosphotransferase system (PTS) A multicomponent system for the transport of carbohydrates into the bacterial cell. The PTS is composed of two general energy-coupling proteins and a set of sugar-specific permeases. The incoming sugars are phosphorylated at the expense of phosphoenolpyruvate concomitant with their transport.
promoter A DNA sequence upstream of a gene that is recognized and bound by the RNA polymerase and used to initiate transcription.
synthetic biology A new field of biology that is devoted to the generation of novel creatures and to the experimental verification of our concepts of life.
terminal organelle (tip structure) A complex cell structure characteristic of the mollicutes. The terminal organelle is required for cell division, movement, and adhesion to the host cell.

Abbreviations

AY-WB	aster yellows *Phytoplasma* strain witches broom
LC	large colony
MMR	multiple mutation reaction
OY-M	onion yellows strain
PCR	polymerase chain reaction
PTS	phosphotransferase system
TCA cycle	tricarboxylic acid cycle

Defining Statement

Mycoplasma and *Spiroplasma* species are bacteria that lack a cell wall (the mollicutes). These organisms evolved in close association with their eukaryotic hosts, resulting in an extreme genome reduction. In this article, the biology of the mollicutes is discussed with special emphasis on their pathogenicity, cell biology, and molecular biology.

Introduction

Mycoplasmas and spiroplasmas are two important genera of the bacterial group called mollicutes. The name mollicutes – *soft skin* – reflects the major collective characteristic of these bacteria – the lack of a cell wall – which at the same time distinguishes them from all other bacteria with the exception of the chlamydiae. The lack of a cell wall is caused by the absence of genes encoding enzymes for peptidoglycan biosynthesis. The lack of a cell wall is closely linked to another characteristic feature of the mollicutes – their cells are usually pleomorphic. Again, there is no rule without exception: the cells of the genus *Spiroplasma* have a helical shape (see following text).

Another important feature of the mollicutes is their close association with eukaryotic host organisms. In nature, mollicutes are never found as free-living organisms. Hosts are either animals including humans (*Mycoplasma*, *Ureaplasma*) or plants and insects (*Spiroplasma*, *Phytoplasma*) (**Table 1**). *Mycoplasma* species usually cause mild diseases such as atypical pneumonia (*Mycoplasma pneumoniae*) or nongonococcal urethritis (*Mycoplasma genitalium*). However, there is an interesting exception: *Mycoplasma alligatoris*, a pathogen of alligators, causes lethal infections. Although the infections caused by mollicutes are rarely lethal, mollicutes pathogenic for plants and animals cause a significant economic loss in agriculture. This is true for cattle in Africa that are infected by *Mycoplasma mycoides* as

Table 1 The systematic groups of the mollicutes

Order	*Genus*	*Genome size*	*Sterol requirement*	*Characteristics*	*Habitat*
Mycoplasmatales	*Mycoplasma*	580–1350 kb	Yes	Growth optimum: 37 °C UGA as Trp codon	Humans, animals
	Ureaplasma	760–1170 kb	Yes	Urea hydrolysis UGA as Trp codon	Humans, animals
Entomoplasmatales	*Entomoplasma*	790–1140 kb	Yes	Growth optimum: 30 °C	Insects, plants
	Mesoplasma	870–1100 kb	No	Growth optimum: 30 °C UGA as Trp codon	Insects, plants
	Spiroplasma	780–2200 kb	Yes	Growth optimum: 30–37 °C UGA as Trp codon Helical motile filaments	Insects, plants
Anaeroplasmatales	*Anaeroplasma*	1500–1600 kb	Yes	Obligate anaerobes	Bovine/ovine rumen
	Asteroleplasma	1500 kb	No	Obligate anaerobes	Bovine/ovine rumen
Acholeplasmatales	*Acholeplasma*	1500–1650 kb	No	Growth optimum: 30–37 °C UGA as stop codon	Animals, plants, insects
	Phytoplasma	640–1185 kb	Not known	Uncultured *in vitro* UGA as stop codon	Insects, plants

well as for rice crops in some regions of Southeast Asia that are infected by phytoplasmas. These losses not only have an economic dimension, but also a significant effect on human nutrition in the affected regions. *Mycoplasma* species such as *Mycoplasma hyorhinis* or *Acholeplasma laidlawii* are major sources of cell culture contamination and have gained increasing interest. These infections are often discovered only late in the course of an experiment and can invalidate the scientific research.

The close association of mollicutes with eukaryotic hosts and their adaptation to habitats with a good nutrient supply and relatively constant growth conditions led to a remarkable process of reductive genome evolution. The organism with the smallest known genome capable of independent life (if provided with rich artificial medium) is *M. genitalium*, a human pathogen. This organism has a genome size of only 580 kb and encodes about 480 proteins, as compared to about 4 million bp and 4000 genes for bacteria such as *Escherichia coli* or *Bacillus subtilis*. These small genomes made the mollicutes important tools for the new discipline of synthetic biology (see 'Genomic comparisons of mollicutes').

The Systematics of the Mollicutes

Evolution of the Mollicutes

The analysis and comparison of 16S rRNA sequences revealed that the mollicutes belong to the Gram-positive bacteria with genomes of low GC content. Ironically, most members of this phylum are characterized by their thick Gram-positive cell wall, and the group is therefore referred to as the firmicutes. This bacterial phylum includes the lactic acid bacteria (such as *Streptococcus* and *Lactobacillus*), spore-forming bacteria (*Bacillus* and *Clostridium*) and their close relatives (*Listeria* and *Staphylococcus*). As can be seen in the phylogenetic tree of the firmicutes (**Figure 1**), the mollicutes form a sister group to the large *Bacillus*/lactic acid bacteria group. It is believed that the first mollicutes emerged some 600 million years ago and that significant loss of ancestral genomic sequences was a major force in the evolution of the mollicutes.

The mollicutes are subdivided in several ways. Three traditional classifications rely on genetic or physiological properties of the bacteria, whereas more recent classification schemes are based on the similarity of the 16S rRNA or conserved protein families.

Two large groups of mollicutes can be distinguished based on their host organisms. Although most mollicutes infect exclusively animal hosts, there are other representatives (*Spiroplasma* and *Phytoplasma*) that are capable of infecting both plant and insect hosts. Another conventional way of classifying the mollicutes is based on their requirement for sterols. Most genera need sterols for growth, whereas this is not the case for the members of the genus *Acholeplasma* (see **Table 1**). However, this requirement can only be determined for those mollicutes that can be cultivated, and many (perhaps most) representatives have not yet been cultured, including all species of the genus *Phytoplasma*. Another peculiarity of most mollicutes is their codon usage: they use the UGA codon to specify tryptophan rather than as a stop codon as in the universal genetic code. Only the genera *Acholeplasma* and *Phytoplasma* among the mollicutes use UGA as a stop codon. Because this is the ancestral property, it can be assumed that *Acholeplasma* and *Phytoplasma* represent the more ancestral mollicutes. This

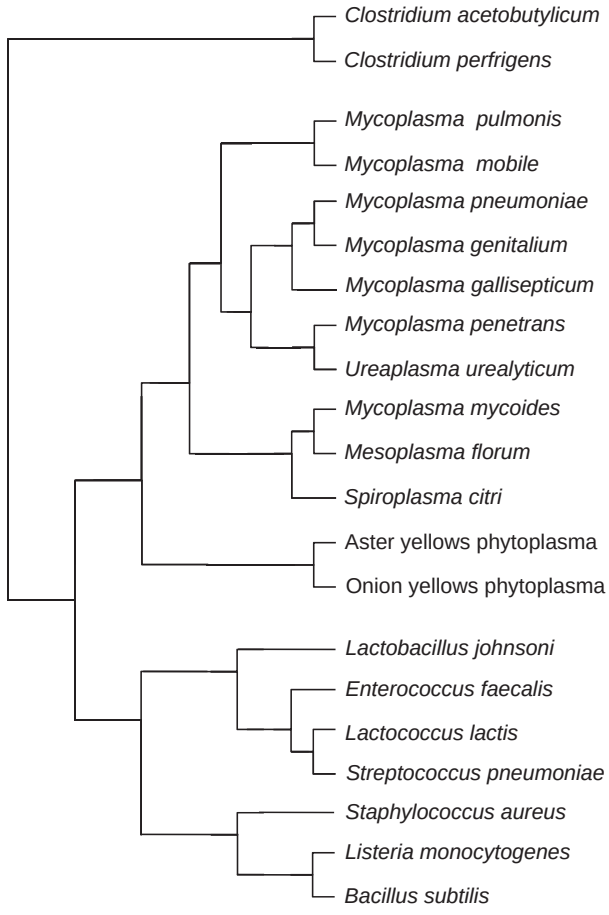

Figure 1 Unrooted phylogenetic tree of the firmicutes with special emphasis to the mollicutes. The tree is based on a concatenated alignment of 31 universal protein families. Reproduced from Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, and Bork P (2006) Toward automatic reconstruction of a highly resolved tree of life. *Science* 311: 1283–1287.

conclusion is supported by a phylogenetic tree based on a concatenated alignment of 30 protein families present in all mollicutes that places the genus *Phytoplasma* at the bottom of the tree (**Figure 1**). The genus *Acholeplasma* is not included in this analysis because of the lack of genome sequence information. It is interesting to note that the genus *Mycoplasma* is paraphyletic, and that genera such as *Spiroplasma*, *Mesoplasma*, and *Ureaplasma* have specific relatives among the different *Mycoplasma* clades (**Figure 1**).

For practical reasons, the mollicutes are grouped in four orders that do not represent the phylogenetic relationships. An overview of these taxa is provided in **Table 1**.

Mycoplasma

As mentioned earlier, the genus *Mycoplasma* is a paraphyletic collection of mollicutes that are widespread in nature as parasites of humans, mammals, birds, reptiles, and fish. The first representative of the genus *Mycoplasma* was identified in 1898 as the causative agent of contagious bovine pleuropneumonia (*M. mycoides*). The human pathogens *Mycoplasma hominis* and *M. pneumoniae* were discovered in 1937 and 1944, respectively. Even now, new species are being identified: in 1981, *M. genitalium* was isolated from a patient suffering from nongonococcal urethritis, and more recently, *Mycoplasma penetrans* and *Mycoplasma fermentans* were found to be associated with HIV infections.

The primary habitats of human and animal mycoplasmas are the mucous surfaces of the respiratory and urogenital tracts, the eyes, the alimentary canal, and mammary glands. In addition, cell cultures are an artificial habitat for many *Mycoplasma* species. The mycoplasmas exhibit a rather strict host and tissue specificity, probably reflecting their highly specific metabolic demands and their parasitic lifestyle. For example, *M. pneumoniae* and *M. genitalium* are preferentially detected in the respiratory and urogenital tracts, respectively.

If cultivated in the laboratory, mycoplasmas as well as other mollicutes require complex media containing sugars, amino acids, nucleotides, and vitamins. It has so far been impossible to cultivate them on chemically defined media.

The complete genome sequences of ten species of the genus *Mycoplasma* have been determined so far. This large interest in the variability of the *Mycoplasma* genetic complement is stimulated by the interest in creating artificial organisms based on the *Mycoplasma* species (i.e., synthetic biology; see following text). The genome sequences revealed the reason for the complex nutritional requirements of the mycoplasmas: they lack the genes for many biosynthetic pathways and are thus dependent on their host or on the artificial medium to provide these required nutrients. Another interesting feature revealed by genome sequences is that only very few known regulatory proteins are present. Again, this is reflective of their close adaptation to one single natural habitat and a result of the reductive evolution: while a metabolically versatile bacterium such as *Pseudomonas aeruginosa* that is capable of thriving in a wide variety of environments reserves as much as 10% of its genome for regulatory genes, only a handful of these genes is found in the mycoplasmas (see following text).

Pathogenicity has been most intensively studied with *M. pneumoniae*. In contrast to most other pathogenic bacteria, *M. pneumoniae* and other mollicutes do not seem to produce any exo- or endotoxins. However, a recent study suggests the formation of a protein similar to ADP-ribosylating and vacuolating cytotoxin. However, this observation has not been confirmed by other groups. A major factor contributing to cytotoxicity and thus to pathogenicity of *M. pneumoniae* is the formation of hydrogen peroxide. The synthesis of hydrogen peroxide by mycoplasmas is most strongly increased if the bacteria are supplied with glycerol. This can be attributed to the oxidase activity of the enzyme that oxidizes glycerol

3-phosphate. This enzyme, glycerol-3-phosphate oxidase, uses water rather than NAD^+ (as in typical glycerol-3-phosphate dehydrogenases) as the electron acceptor. The hydrogen peroxide formed by *M. pneumoniae* acts in concert with endogenous toxic oxygen molecules generated by the host cells and induces oxidative stress in the respiratory epithelium. The effects of the peroxide on the host cells include loss of reduced glutathione, denaturation of hemoglobin, peroxidation of erythrocyte lipids, and eventually the lysis of the cells. Another result of infection by *M. pneumoniae* is the release of proinflammatory cytokines by the host cells. It has been suggested that cytokine production leads to chronic pulmonary diseases such as bronchial asthma.

The significance of glycerol metabolism in hydrogen peroxide production and virulence has been convincingly demonstrated by a series of studies that started with an analysis of the differences between European and African strains of *M. mycoides*, the causative agent of contagious bovine pleuropneumonia. Glycerol transport is highly efficient in the African isolates, whereas it is barely detectable in the European isolates. Because glycerol catabolism gives rise to the formation of hydrogen peroxide, it is not surprising that hydrogen peroxide production is high in the African strains but low in the European isolates of *M. mycoides*. In consequence, the African strains are highly virulent to cattle, whereas their European relatives are harmless. It has been hypothesized that intracellular formation of large quantities of hydrogen peroxide would be toxic for the producing cells themselves. Accordingly, the cellular localization of the responsible enzyme, GlpO, was studied in *M. mycoides* and it was found to be located in the cell membrane. The inactivation of GlpO by antibodies results in the loss of cytotoxicity of *M. mycoides* toward bovine epithelial cells. Given that hydrogen peroxide in concentrations similar to those produced by *M. mycoides* is not cytotoxic, it was concluded that GlpO is not only inserted in the bacterial cell membrane but also in the membrane of the host cell to inject the cytotoxic hydrogen peroxide directly into the epithelial cells. This may cause oxidative stress and subsequent cell death.

Plant Pathogenic Mollicutes: *Spiroplasma* and *Phytoplasma*

The genera *Spiroplasma* and *Phytoplasma* contain plant-pathogenic mollicutes that shuttle between plant and insect hosts. *Spiroplasma citri* was identified in 1971 as a causative agent of citrus stubborn disease. Phytoplasmas were first described in 1967 as the probable cause of plant yellow diseases. Originally, it was speculated that these diseases are of viral origin, and only in 1967 it became clear that these pathogens are *Mycoplasma*-like organisms. While spiroplasmas can be cultivated in the laboratory, no cultivation of any representative of the phytoplasmas has been reported. Therefore, no valid species description for members of the genus *Phytoplasma* is available. Moreover, *Spiroplasma* cells have a spiral morphology, whereas phytoplasmas are pleomorphic.

Spiroplasma species live in the phloem sieve tubes of their host plants. They are transmitted by insect vectors that feed on the phloem sap. Multiplication of the bacteria occurs both in the plant and in the insect hosts. The most intensively studied representative of the genus, *S. citri*, infects periwinkle (*Catharanthus roseus*) and its vector, the leafhopper *Circulifer haematoceps*. Unfortunately, no genome sequences of any *Spiroplasma* species are so far publicly available, although the *Spiroplasma kunkelii* genome has recently been sequenced.

The spiroplasmas are unique among the mollicutes for their helical cell morphology, and also by their unique mechanism of locomotion. The genetic determinants for this distinct morphology and movement are so far unknown. Although the spiroplasmas have a shape that is similar to that of the members of the genus *Spirillum*, they are different because they do not possess flagella. Propulsion is generated by a propagation of kink pairs down the length of the cell, caused by a processive change of cell helicity. In addition, these waves of kinks seem to be initiated always by the same end of the cell suggesting cell polarity. Cell polarity can also be concluded from the results of diverse microscopic studies that showed heterogeneity of both ends: one end is tapered with a tip-like structure called terminal organelle and the other one is blunt or round.

An interesting aspect of the *S. citri* lifecycle is the differential utilization of carbohydrates as source of carbon and energy in the two hosts. *S. citri* possesses the genetic equipment for the utilization of sorbitol, trehalose, glucose, and fructose as carbon sources, which are mainly catabolized to acetate. The two habitats of *S. citri* differ significantly in their carbon source availability. While glucose and fructose are predominant in phloem sieve tubes of plants, trehalose is the major sugar in the hemolymph of the vector insect, the leafhopper *C. haematoceps*. The glucose and trehalose permeases of the *S. citri* phosphotransferase system (PTS) share a common IIA domain encoded by the *crr* gene, which might be involved in the rapid physiological adaptation to changing carbon supplies. The glucose and fructose found in the plant sieve tubes are both derived from the cleavage of sucrose by the plant enzyme invertase. A transposon mutagenesis study with *S. citri* revealed that mutants devoid of a functional *fruR* gene encoding the transcriptional activator of the fructose utilization operon are no longer phytopathogenic. The fructose operon of *S. citri* contains three genes, *fruR*, *fruA*, and *fruK* encoding the transcription activator, the fructose-specific permease of the PTS, and the fructose-1-phosphate kinase, respectively.

Mutations in the *fruA* and *fruK* genes also resulted in decreased phytopathogenicity. However, these mutant strains could revert, and this reversion also restored severe symptoms upon plant infection. Thus, fructose utilization and pathogenicity are intimately linked in *S. citri*. In contrast to mutations affecting fructose utilization, a *ptsG* mutation abolishing glucose transport into the cell does not result in reduced pathogenicity of *S. citri*. The reason for the differential implication of the two sugars in pathogenicity was studied by nuclear magnetic resonance analysis and it turned out that the bacteria use fructose preferentially, whereas the glucose accumulated in the leaf cells of the infected plants. This led to the following model. In noninfected plants, both fructose and glucose are formed by invertase. Fructose inhibits this enzyme resulting in a very low activity. In contrast, no inhibition occurs in infected plants because of fructose utilization by *S. citri*. The accumulating glucose that is not used by the bacteria results in inhibition of photosynthesis and thus in the different symptoms.

Transmission from an infected plant to an insect vector occurs by the uptake of bacteria along with the phloem sap. Inside the leafhopper, the bacteria have to pass the intestine midgut lining to multiply in the hemolymph, and then infect the salivary glands. Infection of the salivary glands is important because transmission from the insect to a host plant occurs by inoculation of the saliva into the damaged plant during feeding. It was shown that certain adhesins are necessary for transmissibility of *S. citri* from an infected plant to a vector, and that the genes coding for these adhesins are located on plasmids not existing in all *S. citri* strains.

In contrast to the spiroplasmas whose members are pathogenic to a broad range of plants and insects, the phytoplasmas form their own group among the mollicutes that is strictly pathogenic to plants. Like the plant-pathogenic spiroplasmas, they inhabit the phloem sieve tubes of their host plants after infection by an insect vector (usually belonging to the family of Cicadelli), but they depend completely on their host and so far it has been impossible to cultivate them *in vitro*. However, the genome sequences of three members of this group, *Candidatus Phytoplasma asteris* onion yellows strain (OY-M), aster yellows *Phytoplasma* strain witches broom (AY-WB), and *Candidatus Phytoplasma australiense* have been determined.

Compared to other members of the mollicutes, the phytoplasmas have some unique features. They exhibit shapes that range from rounded pleomorphic cells, with an average diameter of 200–800 μm, to filaments. Their genomes lack all known genes coding for cytoskeleton or flagellum elements, suggesting that translocation of cells *in planta* is a passive event caused by the flow of phloem sap. As other mollicutes, the phytoplasmas lack genes for the *de novo* synthesis of amino acids, fatty acids, or nucleotides but they also lack some genes considered to be essential in all bacteria, such as *ftsZ* encoding a tubulinlike protein. As FtsZ is involved in cell division, the mechanism of division in the phytoplasmas lacking it must be completely different from that of other bacteria. Although living in an environment that is rich in carbon sources, neither of the sequenced phytoplasma possesses genes coding for sugar-specific components of the PTS. In contrast, *S. citri* and *S. kunkelii*, which thrive in the same environment as the phytoplasmas, contain three PTS for the import of glucose, fructose, and the insect-specific sugar trehalose (see earlier). However, *Phytoplasma* possesses the maltose-binding protein MalE. This protein may bind other sugars as well but genes for enzymes making these sugars available for glycolysis are absent. Sucrose, the main sugar in the phloem sap of plants, could be used as a source of carbon and energy, but in sequenced phytoplasmas the gene for sucrose phosphorylase, which is important for sucrose degradation, is absent or fragmented. In general, phytoplasmas possess fewer genes related to carbon metabolism than the other mollicutes. Energy generation in phytoplasmas seems to be restricted to glycolysis because ATP synthases are absent. OY-M *Phytoplasma* contains a P_{2C}-ATPase, which is common in eukaryotic cells but unique among prokaryotes. Another remarkable feature that makes the phytoplasmas unique among the mollicutes is their ability to synthesize phospholipids, supporting a closer phylogenetic relationship to *Acholeplasma*, which do not require sterols.

Biochemistry of the Mollicutes

Cytology of the Mollicutes

The mollicutes differ from other bacteria not only because they lack a cell wall but also by dint of their small cell sizes. A typical cell of *M. pneumoniae* is 1–2 μm long and 0.1–0.2 μm wide (**Figure 2**). In contrast, a typical rod-shaped bacterial cell (such as *E. coli* or *B. subtilis*) is 1–4 μm in length and 0.5–1 μm in diameter.

The absence of a cell wall has serious consequences for the osmotic stability of the mollicute cells. They are much more sensitive to changes of the osmotic conditions than bacteria possessing a cell wall. The parasitic lifestyle of the mollicutes may be directly related to their osmotic sensitivity: the hosts provide them with osmotically constant conditions that would not be found in the external environment. For example, *M. genitalium* is a parasite of the human urogenital tract, and its transmission by sexual contact ensures minimal exposure of the bacteria to an external, osmotically variable, environment. With the exception of the phytoplasmas and acholeplasmas, the mollicutes are unable to produce fatty acids for membrane biosynthesis and are therefore dependent on exogenously provided fatty acids, which are then used

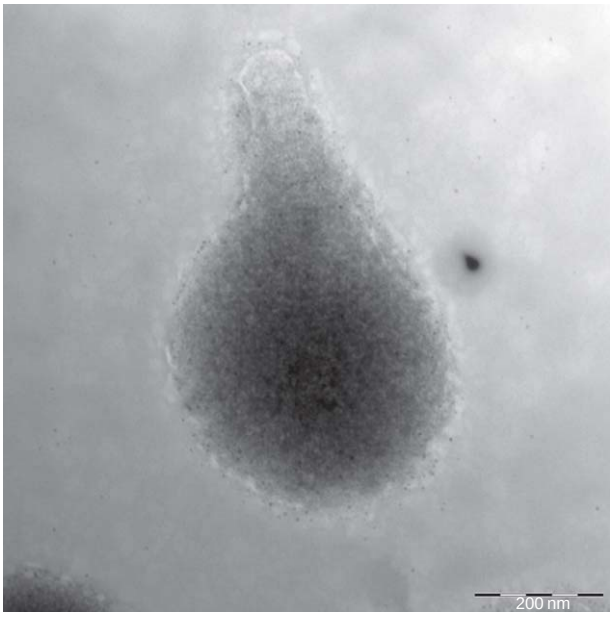

Figure 2 Electron micrograph of a cell of *Mycoplasma pneumoniae*. The terminal organelle (also called the tip structure) is visible in the upper part of the cell. Scale bar = 200 nm.

for phospholipid synthesis. The lack of fatty acid synthesis is accompanied by the absence of a fatty acid desaturase, which is required to adapt the membrane fluidity to lower temperatures. To overcome this difficulty, most mollicutes incorporate large amounts of sterols, which serve as a very effective buffer of membrane fluidity (see **Table 1**).

The lack of a cell wall has also consequences for the cellular morphology of the mollicutes. The cells are pleomorphic; however, they are not small amoebas! The mollicutes exhibit a variety of morphologies, such as pear-shaped cells, flask-shaped cells with terminal tip structures (see below), filaments of various lengths, and in the case of *Spiroplasma* species the cells are helical.

The mycoplasmas have a flask- or clublike shape with a terminal organelle, the so-called tip structure (see **Figure 2**). This tip structure is a complex and specialized attachment organelle that has evolved to facilitate the parasitic existence of the mycoplasmas. The tip structure is made up of a network of adhesins, interactive proteins, and adherence accessory proteins, which cooperate structurally and functionally to mobilize and concentrate adhesins at the tip of the cell. The major adhesin of *M. pneumoniae* is the 170 kDa P1 protein that is responsible for the interaction of the bacteria with the host cells. In addition, the tip structure is important for the internalization of intracellular mollicutes such as *M. penetrans* and *M. genitalium*. *M. penetrans* is capable of actively entering different types of animal cells, even those with minimal phagocytic activity. This may protect the bacterial cells against the host immune system. The formation of the tip structure in *M. pneumoniae* depends on the activity of the P41 protein that serves as an anchor protein. In the absence of this protein, multiple terminal organelles form at lateral sites of the cell and the terminal organelles are not attached to the body of the cell. In *Mycoplasma mobile*, there is also a terminal structure that is referred to as the 'jellyfish' structure made up of a 'bell' with dozens of flexible tentacles. Several components of this structure have been identified. With the exception of the glycolytic enzyme phosphoglycerate kinase, these *M. mobile* proteins are all absent from the genome of *M. pneumoniae* suggesting that the two species found individual solutions for the assembly of the terminal organelle.

Mycoplasma species are able to glide on solid surfaces with the help of their terminal attachment organelle. Terminal organelles that are detached from the body of the *M. pneumoniae* cell are released by some mutants. These detached organelles are still capable of gliding demonstrating that this organelle acts as a novel engine that allows cellular movement. The fastest gliding *Mycoplasma* species, *M. mobile*, contains a dedicated 349 kDa 'leg' protein that is required for gliding. This protein is composed of an oval base with three successive flexible extensions that may support movement. Movement is thought to occur by repeated catching and releasing of sialic acid on solid surfaces and is driven by the hydrolysis of ATP. This ATP hydrolysis may be catalyzed by the glycolytic enzyme phosphoglycerate kinase that is part of the terminal organelle in *M. mobile*.

As other bacteria, the mollicutes divide by binary fission. Again, the terminal organelle seems to be very important for this process: Cell division in *M. pneumoniae* is preceded by the formation of a second tip structure adjacent to the existing one. The two terminal organelles then separate leading eventually to cytokinesis. Among the proteins known to be important for bacterial cell division is the tubulinlike GTP-hydrolyzing FtsZ protein that forms a ring at the division site. Until recently, FtsZ proteins were found in any newly analyzed genome, and the *ftsZ* gene is essential in most bacteria, including *E. coli* and *B. subtilis*. Therefore, FtsZ was considered to be indispensable for all life. However, it recently turned out that some mollicutes such as *M. mobile, Ureaplasma urealyticum*, and the two sequenced phytoplasmas lack *ftsZ* genes, suggesting that its function is dispensable at least in some mollicutes. In many bacteria, the FtsA protein is required for the recruitment of the proteins that form the septum for cell division. Interestingly, this protein is absent from all the pleomorphic mollicutes, whereas it has been detected in *S. kunkelii*. This may be related to the helical morphology of these bacteria.

Metabolism of the Mollicutes

The reductive evolution of the mollicutes is reflected in their limited metabolic properties. Of the central metabolic pathways, that is, glycolysis, the pentose phosphate shunt, and the tricarboxylic acid (TCA) cycle, only glycolysis seems to be operative in most mollicutes. Most striking is the lack of many energy-yielding systems in the mollicutes. No quinones or cytochromes were found in any representative. The electron transport system is flavin-terminated. Thus, ATP is produced by substrate-level phosphorylation, a less efficient mechanism as compared to oxidative phosphorylation.

As observed for *M. genitalium* glyceraldehyde 3-phosphate dehydrogenase, the glycolytic kinases of several mollicute species have functions in addition to that in glycolysis. These enzymes can use not only ADP/ATP but also other nucleoside diphosphate/triphosphate couples. Thus, these enzymes (phosphofructokinase, phosphoglycerate kinase, pyruvate kinase, and acetate kinase) compensate for the lack of the normally essential *ndk* gene encoding nucleoside diphosphate kinase that is required for nucleotide biosynthesis.

Glycolysis is not the only source of ATP formation by substrate level phosphorylation in the mollicutes. Pyruvate can be oxidized to acetyl-CoA by pyruvate dehydrogenase. Acetyl-CoA can be further catabolized by phosphotransacetylase and acetate kinase in an additional substrate level phosphorylation resulting in the formation of acetate. An alternative pathway of pyruvate consumption is its reduction to lactate, leading to the regeneration of NAD^+.

A recent study with *M. pneumoniae* demonstrated that glucose is the carbon source allowing the fastest growth of these bacteria. In addition, *M. pneumoniae* can utilize glycerol and fructose. Interestingly, mannitol is not used even though the genetic equipment to utilize this carbohydrate seems to be complete. Obviously, one or more of the required genes are not expressed or inactive.

Glucose and fructose are transported into the cells by the PTS. This system is made up of general soluble components and sugar-specific membrane-bound permeases. The general components, enzyme I and HPr, transfer a phosphate group from phosphoenolpyruvate to the sugar permease, which phosphorylates the sugar concomitant to its transport.

The arginine dihydrolase pathway can be found also in some *Spiroplasma* and *Mycoplasma* species. Arginine hydrolysis by this pathway results in the production of ornithine, ATP, CO_2, and ammonia. The pathway uses three enzymes: arginine deiminase, ornithine carbamoyl transferase, and carbamate kinase. The degradation of arginine is coupled to equimolar generation of ATP by substrate-level phosphorylation. The role of this pathway as a sole energy-generating source in mycoplasmas is questionable. However, the existence of an arginine–ornithine antiport system in *Spiroplasma melliferum* requiring no ATP for arginine import into the cells supports an energetic advantage in arginine utilization.

Mollicutes possess very limited metabolic and biosynthetic activities for amino acids, carbohydrates, and lipids as compared to 'conventional' bacteria. *M. pneumoniae* scavenges nucleic acid precursors and does not synthesize purines or pyrimidines *de novo*. These may be provided by RNA and DNA that have been degraded by potent mycoplasmal nucleases. Furthermore, both *M. genitalium* and *M. pneumoniae* lack all the genes involved in amino acid synthesis, making them totally dependent on the exogenous supply of amino acids from the host or from the artificial culture medium. The mycoplasmas have also lost most of the genes involved in cofactor biosynthesis; therefore, to cultivate them *in vitro*, the medium has to be supplemented with essentially all the vitamins.

Being dependent on the exogenous supply of many nutrients would predict that mycoplasmas need many transport systems. Surprisingly, *M. genitalium* and *M. pneumoniae* possess a only small number of transport proteins (34 and 44 proteins, respectively) compared to the 281 transport and binding proteins annotated in *E. coli* and almost 400 in *B. subtilis*. The apparent low substrate specificity of some of the mollicute transport systems, such as those for amino acids, may also contribute to the significant gene reduction observed.

Although mollicutes produce hydrogen peroxide, *M. pneumoniae* and *M. genitalium* lack the genes dealing with oxidative stress, such as those encoding catalase, peroxidase, and superoxide dismutase. A thioredoxin reductase system, identified in the mycoplasmas, may protect them from reactive oxygen compounds.

A major problem for the research with mollicutes is the difficulty of cultivating them *in vitro*. Only a minority of the mollicutes existing in nature have been cultivated so far. For example, none of the phytoplasmas infecting insects or plants has been cultivated *in vitro*. To overcome the metabolic deficiencies of the mycoplasmas, complex media are used for their cultivation. The media are usually based on beef heart infusion, peptone, yeast extract, and serum with various supplements. Serum has been shown to provide, among other nutrients, fatty acids and sterols that are required for membrane synthesis. The requirement for sterols has served as an important taxonomic criterion distinguishing the sterol-nonrequiring mycoplasmas, particularly the *Acholeplasma* species, from the sterol-requiring ones. For most mycoplasmas, the pH is adjusted to a slightly alkaline value, conditions that imitate those in the eukaryotic host. A common approach to improve *in vitro* cultivation of fastidious mycoplasmas is based on coculture with eukaryotic cell lines (cell-assisted growth). In this way, some spiroplasmas, such as the Colorado potato beetle *Spiroplasma*, were first successfully cocultivated with insect cell lines.

Genetics and Molecular Biology of the Mollicutes

Gene Expression in the Mollicutes

The basic mechanisms of gene expression have been studied poorly in the mollicutes. They possess a conventional bacterial RNA polymerase, but unlike most other bacteria, they encode only one sigma factor of the RNA polymerase. Thus, diversity of promoters and RNA polymerase holoenzymes are not used for regulatory purposes in the mollicutes. The transcription start sites have been identified for several *M. pneumoniae* genes, and it turned out that the −10 region of these promoters is similar to that recognized by the housekeeping sigma factors of other bacteria such as *E. coli* or *B. subtilis*. In contrast, there is no conserved −35 region. These observations were confirmed by a recent analysis of the sequence determinants that are required for promoter activity in front of the *M. pneumoniae ldh* gene encoding lactate dehydrogenase. The −10 region is essential for transcription initiation, whereas the −35 region could be mutated without any consequences. Thus, the single *M. pneumoniae* RNA polymerase holoenzyme recognizes only the −10 region for promoter recognition.

Another peculiarity of the *M. pneumoniae* transcription machinery is the lack of the termination factor Rho, and correspondingly, the absence of Rho-dependent transcription terminators. Surprisingly, a bioinformatic analysis of bacterial genomes and the free energy values of RNAs around the end of open reading frames suggest that the mollicutes do also not contain functional Rho-independent transcription terminators. This raises the important question of how transcription is terminated in the mollicutes or whether it is terminated at all. The answer came from Northern blot experiments aimed at the identification of *in vivo* transcripts, and this answer is ambiguous. Indeed, defined transcripts were observed in a few cases, such as the *M. genitalium* and *M. pneumoniae ftsZ* gene clusters or the *M. pneumoniae ptsH* gene. The existence of these defined transcripts implies that there are also defined transcription terminators present. However, these terminators may be very rare. This might explain the observation that unrelated genes are expressed as parts of one transcription unit in the mollicutes. Moreover, most attempts to determine transcript sizes by Northern blot analysis in the mollicutes have failed. This is probably the result of mRNA length polymorphisms, which prevent the detection of clearly defined RNA species.

Most genes in the mollicutes have the same orientation on the chromosome, and the intergenic regions are usually quite short if present at all. The transcription of most of these large gene clusters is colinear with replication. This genome organization also favors polycistronic transcription of large gene clusters.

The lack of defined mRNA species results not only from the absence of transcription terminators but also from the weak conservation of sequences that mediate transcription initiation: a −10 region made up of only Ts and As is statistically overrepresented in the AT-rich mollicute genome. Indeed, the −10 regions predicted from the analysis of many start points occur about 2900 times in the 816 kb genome of *M. pneumoniae*. This large number of possible transcription initiation sites is also reflected by the observation of substantial antisense transcription in both *M. genitalium* and *M. pneumoniae*.

In bacteria, regulation is usually exerted at the level of transcription. In the mollicutes, only one example of transcription regulation is clearly documented: this is the regulation of the *S. citri* fructose operon by the transcription activator FruR (see earlier text). Moreover, the induction of chaperone-encoding genes at elevated temperatures was demonstrated in several *Mycoplasma* species. By analogy to the mechanism of heat shock regulation by the repressor protein HrcA and the DNA operator element CIRCE, it was proposed that heat shock genes are under the control of HrcA in the mollicutes. In addition to HrcA, the genomes of *M. genitalium* and *M. pneumoniae* encode only two other potential transcription factors that belong to the GntR and the Fur family, respectively. Unfortunately, the function of these regulators has so far not been studied.

It is interesting to note that *M. pneumoniae* contains only three potential regulators (less than 0.5% of all open reading frames), whereas environmental bacteria such as *Streptomyces coelicolor* and *P. aeruginosa* reserve about 10% of their genetic capacity to encode transcription factors. The low number of transcription factors in the mollicutes and the weak stringency of transcription signals in the mollicutes might therefore reflect their close adaptation to specific habitats that provide a good supply of nutrients and protect the bacteria from harmful environmental conditions. Moreover, the good supply of nutrients from external sources, that is, the host, may abolish the need for transcription regulation, that is, to switch off the expression of genes if their products are not required.

An additional mechanism of regulation is provided by riboswitches and regulatory RNAs. A guanine-specific riboswitch was detected in the untranslated region of the *Mesoplasma florum guaAB* operon suggesting that this RNA element governs the regulation of this operon via guanine.

Translation is one of the most prominent activities of the mollicute cell: as much as 15% of the genome of the mollicutes is devoted to translation-related functions. The principal mechanisms of translation in the mollicutes are identical to those found in other bacteria. Because of the low genomic GC content, the codon usage is strongly biased toward AT-rich codons. With the exception of

Phytoplasma and *Acholeplasma*, the mollicutes decode the UGA codon as tryptophan instead of using it as a stop codon as in the universal genetic code. This poses severe problems for the expression of mollicute proteins in heterologous hosts (see following text).

The mechanisms of translation initiation seem to differ among the mollicutes. In some organisms such as *Mycoplasma capricolum* and *S. citri*, the open reading frames are preceded by canonical Shine–Dalgarno sequences that form base pairs with the 3′ end of the 16S rRNA. In contrast, many genes of *M. pneumoniae* and *M. genitalium* lack such a sequence, and moreover, leaderless mRNAs are common in these bacteria. The molecular mechanisms of translation initiation in *M. pneumoniae* and its close relatives still await elucidation.

Posttranslational Protein Modification

In many bacteria including the mycoplasmas, the HPr protein of the PTS cannot only be phosphorylated by enzyme I but is also the target of a regulatory phosphorylation on Ser-46 by a metabolite-activated protein kinase, HPrK. The phosphorylation of HPr on Ser-46 in 'less degenerated' firmicutes leads to carbon catabolite repression. So far, the functions of HPrK and ATP-dependent phosphorylation of HPr have not been studied in the mollicutes. In contrast, much work has been devoted to the biochemical characterization of HPrK from *M. pneumoniae*. Unlike its equivalent from other bacteria, this protein is active at very low ATP concentrations. As in related proteins, it contains an essential Walker A motif for ATP binding. Mutations in this region severely affect both the kinase and the phosphatase activities of the protein. Fluorescence studies revealed that the *M. pneumoniae* HPrK has a significantly higher affinity for ATP than any other HPrK studied so far. This may explain why it is active even at low ATP concentrations. The *M. pneumoniae* HPrK was crystallized and its structure determined. As observed for homologous proteins, it forms a hexamer with the C-terminal domains in the active center.

In addition to HPrK, there is one other protein kinase in *M. pneumoniae* and many other mollicutes, PrkC. The corresponding gene is clustered with the gene encoding a protein phosphatase of the PP2C family, PrpC. It was shown that PrpC is implicated in the dephosphorylation of HPr(Ser-P). PrkC is known to phosphorylate a wide variety of proteins in other firmicutes; however, its targets and the role of PrkC-dependent phosphorylation in the mollicutes remain to be studied.

Protein phosphorylation seems to be important for the biology of the mollicutes. An analysis of the *M. genitalium* proteome revealed that each identified protein is present at an average of 1.22 spots on a 2-D gel, suggesting posttranslational modification of about 25% of all proteins. Given the importance of protein phosphorylation in all other living organisms, it seems safe to assume that a large portion of these modified proteins is actually phosphorylated. A phosphoproteome analysis of *M. genitalium* and *M. pneumoniae* identified 5 and 3% of the total protein complement of these bacteria, respectively, as phosphoproteins. Among these proteins are not only enzymes of central carbon metabolism such as enolase and pyruvate dehydrogenase subunits, but also several cytoskeleton and cytadherence proteins. It is tempting to speculate that PrkC may catalyze these phosphorylation events.

As in other bacteria, there is protein secretion in the mollicutes. While some exported proteins carry typical signal peptides at their N-termini, there is no signal peptidase I present in the genome of the mollicutes. This raises the possibility that so far uncharacterized proteins are active in protein secretion in the mollicutes.

Genomic Comparisons of Mollicutes

One of the questions that have been of interest to humans since its early days is the problem of what constitutes life. Only today, in the era of genome research, are we able to attempt an answer to this question. A major milestone in defining life was the identification of key features that characterize all living things and differentiate them from nonliving matter such as viruses and prions. Among these features are metabolism, autonomous replication, communication, and evolution. With the availability of genome sequences, it is has become possible to determine the genetic equipment required for independent life. The mollicutes are of special interest in this respect because they have the smallest genomes that allow independent life, at least under laboratory conditions.

Genome research with the mollicutes is driven by two major challenges: (1) the identification of the minimal set of genes that is required for independent life and (2) the creation of artificial organisms that are based on this minimal gene set. The simplicity of the mollicutes and the broad body of knowledge on their biology makes them ideal starting points for these research areas.

Several different strategies have been applied to identify the minimal gene set required for life. The most simple approach is based on the comparison of sequenced genomes of different organisms. It seems safe to assume that those genes that are conserved in different organisms are more important than those that appear only in certain species. The smallest genome of any independent living organism known so far is that of *M. genitalium*. This bacterium has a genome of 580 kb with 482 protein-coding genes and 39 genes coding for RNAs. *M. pneumoniae* has a genome of 816 kb with 779 genes coding for proteins and 40 RNA-coding genes. A comparison of the two genomes reveals an overlap of 477 genes common to both species. This suggests that *M. pneumoniae* is an 'extended version' of *M. genitalium*. It is tempting to

speculate that *M. genitalium* is further advanced on the pathway of reductive genome evolution. Indeed, some genes present in *M. pneumoniae* but not in *M. genitalium* such as the mannitol utilization genes are known to be nonfunctional in the former organism. Thus, *M. genitalium* seems to be very close to a true minimal organism.

A comparison of all sequenced mollicute genomes reveals that only a small subset of their genes is part of a common gene pool. Only 156 genes are common to all mollicute genomes that have so far been sequenced. This represents about one-third of the 482 open reading frames of *M. genitalium*. Interestingly, of the 156 genes of the mollicute core genome, the large majority, that is, 124 genes, are shared by all firmicutes. Thus, there is only a small set of 32 genes that is conserved in all mollicutes but not in all firmicutes. However, even these genes are shared by many members of the firmicutes thus precluding the idea of a gene set unique to the mollicutes. Moreover, a large fraction of the common mollicute gene set forms the core genome of all bacteria (about 100 genes). Thus, the genome reduction of the mollicutes obviously went down to a minimum that is absolutely required for cellular life. This is becoming clear if one takes into account that even unrelated bacteria such as *E. coli* (γ-proteobacterium) and *B. subtilis* (firmicute) share about 1000 genes.

The core gene set of the mollicutes is made up mainly of genes encoding proteins involved in essential cellular functions such as DNA topology, replication and repair, transcription, RNA modification and degradation, translation, protein folding, secretion, modification, or degradation (**Table 2**). In addition, seven genes encoding potential GTP-binding proteins are conserved in all mollicute genomes. A few conserved metabolic genes encode proteins involved in glycolysis, metabolite and ion transport, nucleotide, lipid, phosphate, and amino acid metabolism. Interestingly, not a single protein of completely unknown function is conserved among all mollicutes. Moreover, the genes common to all mollicutes act in the central processes of life. This implies that there are no genes common to all mollicutes that are required for mollicute-specific activities such as the formation of the terminal organelle. This is in good agreement with earlier studies that demonstrated a large variability in the protein composition of this organelle.

A second approach to determine the minimal gene set required for life uses an experimental setup. Global transposon mutagenesis studies with *M. genitalium* and *M. pneumoniae* revealed dispensable genes. For *M. genitalium*, about 100 genes could be disrupted. This implies that the remaining 382 genes are essential. In addition, five genes that are part of groups of redundant genes seem to be essential. It is believed that these 387 genes (plus the RNA-coding genes) constitute the essential gene set of *M. genitalium*. The difference between the 156 genes in the core gene set of the mollicutes and the 387 genes that are essential for *M. genitalium* suggests that many of the additional genes are important under the specific ecological conditions of *M. genitalium*. This idea is supported by the presence of 110 genes of unknown function among the essential genes. This finding clearly demonstrates how much remains to be learned about the biology of *M. genitalium*, and surely about the other mollicutes as well.

Table 2 The core gene set of the mollicutes

Function	*Number of genes*
Information pathways—Protein	
Ribosomal proteins	38
Translation factors	11
Amino acyl tRNA synthetases	19
Chaperones	2
Proteolysis	3
Protein modification	1
Protein secretion	5
Information pathways—RNA	7
Transcription	7
RNA modification	8
RNA degradation and maturation	5
Information pathways—DNA	
Replication	7
Repair	8
DNA topology	3
Metabolism	
Basic carbon and energy metabolism	8
Amino acid metabolism	1
Nucleotide biosynthesis	6
Pyrophosphatase	1
Lipid metabolism	1
Miscellaneous functions	
Transport	7
GTP-binding proteins	7
Unknown proteins	7 (mge_009, 056, 132, 222, 366, 505, 516)

With information on the minimal gene set in hand, the logical next step will be to construct artificial organisms with this set of genes. In 2007 and 2008, two important technological steps have been made on the way to the construction of such minimal artificial life: first, the replacement of one genome by another, a process called genome transplantation, was demonstrated. Genomic DNA of *M. mycoides* large colony (LC) was used to replace the genome of *M. capricolum* by polyethylene glycol-mediated transformation. The second major achievement was the chemical synthesis and assembly of the *M. genitalium* chromosome. Thus, an artificial chromosome can be synthesized and this DNA can be introduced into a living cell to provide the environment for the expression of this genome. The generation of an artificial minimal *Mycoplasma*-derived organism ('*Mycoplasma laboratorium*') would be the logical

next step and the ultimate proof of both these technologies and of our understanding for the minimal equipment of a living cell.

Molecular Biology and Genetic Tools for the Mollicutes

The detailed genetic analysis of the mollicutes has been hampered for a long time by the lack of genetic tools that allow the efficient expression of UGA-containing mollicute genes in heterologous hosts for purification and subsequent biochemical analysis, the stable introduction of foreign genetic material into a mollicute cell, and either the targeted construction or the targeted isolation of desired mutant strains. During the past few years considerable progress has been made in the field of mollicute genetics, making these organisms accessible for genetic studies.

The occurrence of UGA codons in the genes of mollicutes has often prevented their expression in heterologous hosts for detailed biochemical analysis, because they serve as stop codons in *E. coli* and other expression hosts. To circumvent this problem, a variety of different but rather dissatisfying strategies had been employed, including the expression of UGA-containing genes in opal suppressor strains of *E. coli*, or in *S. citri* that also reads UGA as a tryptophan codon. As long as only few UGA codons are present in a gene, their sequential replacement by standard site-directed mutagenesis strategies might also be taken into consideration. However, the latter approach is time-consuming and cost-intensive with an increasing number of UGA codons. Recently, a strategy referred to as multiple mutation reaction (MMR) allowing the simultaneous replacement of multiple UGA codons in a single-step reaction was developed. This strategy is based on the use of 5′-phosphorylated oligonucleotides containing the desired mutations in a polymerase chain reaction (PCR). During the elongation steps, the external amplification primers are extended. As the mutation primers are designed to hybridize more strongly to their targets, the elongated amplification primers can then be ligated to the 5′ ends of the mutation primer by a thermostable DNA ligase, yielding a DNA strand that contains the desired mutation. With this strategy, the simultaneous introduction of up to nine mutations in one single step is possible.

The majority of genetic tools that are well established in model organisms are unavailable for mollicutes. Therefore, transposons are in common use for a variety of purposes. In combination with smart screening systems, they were used for the disruption of genes but also as carriers for the introduction of genetic material into the chromosome. The transposons Tn*916* and Tn*4001* and their improved derivatives can be used in mollicutes. These transposons were originally isolated from *Enterococcus faecalis* and *Staphylococcus aureus*, respectively, and have a broad host range. Tn*916* is a conjugative 18 kb transposable element that contains the *xis-Tn*/*int-Tn* genes for excision/integration, followed by the *tetM* tetracycline resistance determinant and a set of genes (*tra*) required for intercellular transfer. Tn*916* does not generate target duplications at its integration site, because it transposes by an excision/integration mechanism that is based on staggered nicks in the donor DNA. Tn*4001* is a 4.5 kb composite transposon consisting of two identical IS*256* elements flanking the gentamicin/kanamycin/tobramycin resistance conferring *aac-aphD* gene. Tn*4001* has been used for transforming several *Mycoplasma* species. To increase the stability of transposon insertion mutants, mini-transposons on the basis of Tn*4001* were constructed that have the transposase gene outside the transposable elements to prevent reexcision of the transposon after the first transposition event.

Until very recently, the targeted construction of gene knockout mutants via homologous recombination has only been reported in a few mollicutes such as *M. genitalium*, *Mycoplasma gallisepticum*, *Mycoplasma pulmonis*, and *A. laidlawii*. In the absence of homologous recombination, the only remaining way to obtain gene knockouts is transposon mutagenesis. Because of the randomness of integration, the screening of large transposon mutant libraries for the loss or gain of a specific phenotype is required to isolate a gene knockout of interest. If no screenable phenotype can be expected to be associated with a gene of interest, the only known feature of the desired gene knockout is the specific DNA junction between the gene of interest and the transposon. Based on this idea, a strategy referred to as 'haystack mutagenesis' has been designed that allows the targeted isolation of any viable transposon insertion strain out of an ordered library of transposon mutants. The concept of haystack mutagenesis is based on a saturating transposon mutagenesis to ensure that each dispensable gene is disrupted at a desired confidence level. Once the required number of transposon mutants has been isolated, they are arranged in pools of a reasonable size. These pools can then be screened by PCR using a gene-specific oligonucleotide and another one specific to the transposon for identifying the pool that contains the desired insertion. Subsequently, a similar screen at the level of the individual clones of the positive pool will identify the mutant of interest. This strategy has already been used for the isolation of several *M. pneumoniae* mutants. Alternatively, transposon mutant libraries can be screened for mutants that exhibit an interesting phenotype, such as loss of gliding motility.

The use of transposons is accompanied by the problem of changes of the genetic context at the site of integration that may cause undesired side effects. To avoid this problem, autonomously replicating plasmids have always been the vehicle of choice. Some early studies reported the isolation of naturally occurring plasmids from *M. mycoides*. These are small cryptic plasmids with a size

in the range of 1.7–1.9 kb coding for replication functions only. Based on one of these plasmids, *M. mycoides–E. coli* shuttle vectors were developed. Further developments of artificial plasmid vectors were stimulated, when the first genome sequences became available that allowed the determination of the origins of replication of *Mycoplasma* chromosomes. Plasmid replicons have been constructed that contain the *oriC* sequences from *M. mycoides*, *M. capricolum*, and *Mycoplasma agalactiae*. Remarkably, a certain host specificity was observed for *oriC* plasmids, hampering the prediction the *oriC* compatibility between different *Mycoplasma* species and the derived plasmids. Nevertheless, with the genome sequence of many mycoplasmas at hand, the construction of stably replicating *oriC* plasmids for any desired *Mycoplasma* can be expected in the near future.

In the past there have been a couple of studies aimed at the definition of mycoplasmal promoters. The lack of clarity concerning the nature of gene expression/regulation signals in mollicutes (see 'Gene expression in the mollicutes') can only be answered in experiments that make use of promoter reporter systems. Such reporter systems based on the promoterless *lacZ* gene or on fluorescent proteins have been developed and used. They are used in two ways: the reporter genes can be randomly introduced into the chromosome to isolate random fusions with promoters; alternatively, the fusions can be prepared on plasmid vectors before their introduction into the genome. This second possibility allows the analysis of mutant promoter variants.

At present all required tools for the application of standard genetics to mycoplasmas are available. The biochemical *in vitro* analysis of individual proteins is no longer hampered by the genetic code of these organisms. Thus, interesting proteins can be easily studied. Similarly, antigenic surface proteins, which are often very large and thus contain many UGA codons can now easily be produced in heterologous hosts in sufficient amounts to be tested as vaccine candidates. Using the existing reporter systems, it will be possible to refine the mycoplasmal promoter concept, to discover regulatory DNA sequences and, ultimately, unravel the signal transduction mechanisms that mediate the adaptive responses seen in a wide variety of DNA microarray analyses but which are not yet understood at the molecular level. To confirm *in vitro* findings with purified proteins, targeted disruption of desired genes can presently be carried out in various representatives of the genus *Mycoplasma*, either by homologous recombination or by facilitated screening methods such as haystack mutagenesis.

Accompanying Feature

Additional resources on the mollicutes (key references, genome information, labs working on the mollicutes, information on important methods) can be found on an accompanying web page (http://tinyurl.com/3vw8ca).

See also: Adhesion, Microbial; *Bacillus Subtilis*; Cell Structure, Organization, Bacteria and Archaea; Emerging Infections; Genetics, Microbial (general); Phylogenomics

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