Peculiar properties of mycoplasmas: The smallest self-replicating prokaryotes

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Received 23 June 1992
Accepted 3 July 1992

Key words: Mycoplasma cell biology; Mycoplasma pathogenicity; AIDS; Rheumatoid arthritis

1. SUMMARY

Mycoplasmas are the smallest and simplest prokaryotes capable of self-replication, with information provided by a genome which may be as small as 600 kb, estimated to carry less than 500 genes. Keeping the number of structural elements, metabolic pathways and components of the protein synthesizing machinery to an essential minimum places mycoplasmas closest to the concept of 'minimum cells'. Mycoplasmas are, therefore, most adequate candidates for the complete deciphering of the machinery of a self-replicating organism, and studies towards this goal are already underway. Living as 'minimum cells' was made possible by adopting a parasitic mode of life, securing from the host the many nutrients which cannot be synthesized by the mycoplasmas themselves. When pathogenic, infections by mycoplasmas usually follow a chronic course, with host immune reactions playing an important role in symptom production. Recent studies on the possible association of mycoplasmas with rheumatoid arthritis and AIDS are reviewed.

2. INTRODUCTION

The nature of mycoplasmas has presented a continued enigma to microbiologists, ever since the first mycoplasma was discovered in 1898. At the beginning of this century, mycoplasmas were considered to be viruses, as they passed through filters blocking the passage of bacteria. Later on, the mycoplasmas were confused with bacterial L-forms, leading to heated controversies that came to an end only in the late 1960s, when genomic analysis ruled out any relationship of mycoplasmas to L-forms of present-day walled bacteria [1]. The remarkable structural simplicity and small genome size of mycoplasmas led Morowitz and Wallace to propose in 1973 [2] that mycoplasmas are the most primitive extant organisms that should be placed at the root of the phylogenetic tree. This hypothesis lost ground in the early 1980s with the introduction by Carl

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Woese of ribosomal RNA sequences as phylogenetic markers. Accordingly, the mycoplasmas are the product of reductive evolution stemming from the Gram-positive bacillus-lactobacillus-streptococcal lineage, forming a relatively superficial branching within the bacterial tree [3,4]. Yet, phenotypically, mycoplasmas exhibit unique properties, making these organisms most useful models in studying basic problems in cell biology. In fact, recognition that mycoplasmas, despite their idiosyncrasies, can be considered as ordinary eubacteria, supports their use as paradigm organisms.

Since a major goal of this brief review is to emphasize the usefulness of mycoplasmas as model organisms, the review will be very selective rather than exhaustive and systematic. A volume containing an extensive up-to-date coverage of the cell biology and pathogenicity of mycoplasmas is scheduled to appear by the end of 1992 [5].

3. MYCOPLASMAS AS 'MINIMUM CELLS'

The mycoplasmas are the smallest self-replicating organisms. With a diameter as small as 0.3 μm, some mycoplasma cells are not much larger than the theoretical 'minimum cell' [6,7]. In line with their minute size, mycoplasmas carry the smallest genomes recorded in prokaryotes, ranging from about 600 kb to about 1700 kb, depending on species and strain [8–11]. Assuming that 20–30% of the genome consists of intergenic spacers, transcription signals etc., the estimated number of genes in a mycoplasma with a 600–800 kb genome is only 400–500 [12], about one-fifth of the estimated number of genes in Escherichia coli. Two-dimensional polyacrylamide gel electrophoresis detected in M. capricolum, a mycoplasma with a 1155 kb genome [13], about 350 different polypeptides compared to over 1100 polypeptides detected in E. coli [14]. How can an organism with a set of 400–500 genes grow and replicate? The answer appears to lie in the extreme simplicity of mycoplasma cells. These organisms are essentially built of the minimum number of organelles for growth and reproduction: a circular double-stranded DNA molecule providing the genetic information, ribosomes upon which cell proteins are assembled, and a plasma membrane separating the cytoplasm from the external environment.

It can be hypothesized that during the presumed reductive or degenerative evolution of mycoplasmas from the ancestor Gram-positive walled bacterium [3], mycoplasmas lost the cell wall and many biosynthetic systems, apparently by adopting a parasitic mode of life. All known mycoplasmas are parasites of humans, vertebrates, plants and arthropods. Living in the relatively constant environment of their host, mycoplasmas could do without the protection of a rigid cell wall. In this way the considerable number of genes involved in synthesis of the bacterial wall polymers were eliminated. On becoming parasites, mycoplasmas could do without many biosynthetic and degradative pathways and thus do without a considerable number of genes. In fact, mycoplasmas depend on their host for essentially the complete spectrum of amino acids, fatty acids, cholesterol and vitamins, as reflected by their very complex nutritional requirements and by the extreme difficulty in compounding defined culture media for mycoplasmas [15,16]. We have failed in many cases to imitate the nutritional milieu provided by the host, so that many mycoplasmas existing in nature have not been cultivated so far, most prominent in this respect being the plant pathogenic mycoplasma-like organisms [10,17]. Mycoplasmas also lack major energy-yielding systems. Thus, none of the mycoplasmas characterized so far possess the tricarboxylic acid cycle [18]. While many mycoplasmas have the ubiquitous glycolytic system, some lack it, becoming dependent on less effective energy-yielding systems. Thus, some mycoplasmas acquire ATP through arginine hydrolysis by the 3-enzyme arginine dihydrolase pathway [19]. Ureaplasma, lacking both the glycolytic and arginine dihydrolase pathways, developed a unique mechanism by which ATP is produced following the intracellular hydrolysis of urea by the organism's potent urease and the formation of a proton gradient across the cell membrane [15,20]. Obviously, the simple energy-yielding pathways in mycoplasmas signify a considerable saving in genes.
As could be expected, the mycoplasmas have retained the essential protein synthesis system, but the theme of saving on genetic information is also noticeable here. Thus, mycoplasmas carry only one or two ribosomal RNA gene copies compared to seven in *E. coli* and ten in *B. subtilis* [21–24]. The number of tRNA species had also been cut down. Thus, the *M. capricolum* genome carries 30 tRNA genes for 29 tRNA species, compared to 78 tRNA genes in *E. coli* for 45 tRNA species, and 51 genes for 31 different tRNA species in *B. subtilis* [25,26]. This marked saving in genetic information was made possible by a single mycoplasmal tRNA reading an entire family box codon, without discrimination between the nucleotides in the third codon position [27]. In addition, mycoplasmal tRNAs are characterized by having fewer modified nucleosides, saving on modification enzymes and consequently on genomic information [22]. Resembling Gram-positive bacteria, mycoplasmas carry only one copy of the *tuf* gene, coding for the elongation factor EF-Tu, while Gram-negative bacteria carry two copies of this gene [28]. Saving on genes is also expressed with mycoplasmal DNA polymerases. *Mycoplasma* and *Ureaplasma* species have only one DNA polymerase, while *Acholeplasma* and *Spiroplasma* species (which have larger genomes of 1600–1700 kb), possess three distinct DNA polymerases, resembling *E. coli* and other eubacteria [29].

A marked saving in genes in mycoplasmas is manifested with regard to membrane lipid synthesis. *Mycoplasma*, *Ureaplasma* and *Spiroplasma* species cannot synthesize fatty acids and require their exogenous supply [15]. Some mycoplasmas, like *M. gallisepticum*, even require preformed phospholipids for growth [30]. Apart from *Acholeplasma* and *Astereplasma* species, all other cultured mycoplasmas require cholesterol for growth. Dependence on exogenous fatty acids and cholesterol facilitates the introduction of controlled alterations in membrane lipid composition, rendering mycoplasmas most useful models in studying the role of lipid components in biological membrane structure and function [31–34].

Being the simplest self-replicating organisms, mycoplasmas have been proposed by Morowitz [7] to serve as the paradigm organisms for the complete deciphering of the machinery of a living cell. Morowitz expects in this way to prove the dogma of the completeness of molecular biology, i.e. that the logic of life is finite, relatively simple, and subject to full exploration. He proposed the launching of an international effort to accomplish this very ambitious goal. The project requires an enormous amount of work and generous funding, but it does not appear to involve conceptual difficulties, and the methodology required is at hand. The effort should basically consist of the following steps: the selection of the most appropriate mycoplasma, preferentially one which is capable of growth in a defined medium to facilitate construction of metabolic maps and transport systems; the physical and functional mapping of the genome, to be followed by its complete sequencing; establishment of the complete spectrum of metabolic maps and transport systems; definition and characterization of the cytoskeletal elements responsible for cell shape, cell division and motility; and finally, the complete assignment of coding space to structure and function of the cells. Although a collaborative international project has not been organized as yet, significant strides toward reaching the above objectives have already been made. The complete sequencing of mycoplasma genomes is underway. The group of P.M. Gillevet of the Harvard Genome Project is sequencing the *M. capricolum* genome. Richard Herrmann’s group in Heidelberg has initiated the sequencing of the *M. pneumoniae* genome, following its physical mapping [11,35], and the laboratory of J.M. Bove in Bordeaux has been mapping the *S. citri* genome [36,37]. A voluminous amount of data has been accumulating recently concerning specific genomic sequences, mostly those carrying conserved genes, such as the genes for ribosomal proteins and rRNAs, tRNAs, elongation factors, the proton-translocating ATPase, DNA gyrase, RNA polymerase and genes of some key enzymes in eubacterial cell metabolism, [11,13,37–44]. The use of conserved genes of eubacteria (mostly of *E. coli*) as probes in cloning the homologous mycoplasmal genes, has been
most useful in ‘fishing’ for mycoplasmal genes, enabling their sequencing and localization on the mycoplasmal genomic maps.

Genomic information has also been secured through the extensive studies on the adhesins and attachment organelles of the human pathogens *M. pneumoniae* and *M. genitalium*. Since adhesion of these pathogenic mycoplasmas to their target tissue is essential for colonization and parasitism, a significant number of genes had been invested in producing a special attachment organelle at the tip of the flask-shaped cells, consisting of cytoskeletal elements and specific membrane proteins functioning as adhesins [45]. Interestingly, the *M. pneumoniae* and *M. genitalium* genomes, despite being among the smallest recorded (800 kb and 600 kb, respectively) contain a significant number of repeated adhesin gene sequences, estimated to consist of up to 6% of the *M. pneumoniae* genome [45,46]. Another class of genes, rather common in pathogenic mycoplasmas, are those involved in antigenic variations (see Section 4). Clearly, by becoming dependent on host-derived nutrients, a considerable amount of genetic information can be saved. However, there is apparently no ‘free lunch’, since adapting to a parasitic mode of life imposes some demands on genomic information required for specialized attachment structures, adhesins, modification of surface antigens etc., in addition to the need for extra systems to transport the many nutrients provided by the host through the mycoplasma membrane.

### 4. MYCOPLASMAS AS PATHOGENS

Most mycoplasmas live as commensals, and in many arthropods they may even be considered as symbionts [47,48]. In the case of pathogenic mycoplasmas, infections are rarely of the fulminant type, but rather follow a chronic course. It could be argued that mycoplasmas are close to the concept of ‘ideal parasites’, usually living in harmony with their host. Mechanisms of mycoplasma pathogenicity are largely unknown. Mycoplasmas in humans and animals are mostly surface parasites, colonizing the epithelial linings of the respiratory and urogenital tracts. Potent toxins have not been associated with mycoplasmas. In order to explain tissue damage, the mildly toxic by-products of mycoplasma metabolism, such as hydrogen peroxide and superoxide radicals, have been incriminated as causing oxidative damage to the host cell membrane [49]. Another possible mechanism of pathogenicity, proposed but not definitely proved, is based on the intimate association of the wall-less mycoplasmas with the host cell membrane. The close contact may lead to local, perhaps transient, fusion of the two membranes, or to exchange of membrane components, possibilities which are intriguing and should be investigated [45,50,51]. The recent discovery of a new human mycoplasma, *M. penetrans*, capable of active penetration into cells [52,53] as well as the marked invasiveness and the intracellular location of *M. fermentans* (strain incognitus) in AIDS patients [54] and in cell cultures [55] open new ways for explaining mycoplasma pathogenicity. The dogma that human and animal mycoplasmas are exclusive extracellular parasites, with intracellular parasitism being limited to plant and insect mycoplasmas, should be reconsidered in light of the new findings.

Host immune reactions appear to play a major role in pathogenesis of mycoplasma infections. It has been postulated that in *M. pneumoniae* pneumonia in humans, host reactions may be responsible to a large extent for the respiratory symptoms, through monitoring a local immunocytic response to the mycoplasmas [56]. Moreover, the post-infection sequelae of *M. pneumoniae* pneumonia, affecting the central nervous system, the blood, skin and other organs, have been attributed to autoantibodies induced during infection. Best characterized are the cold agglutinins directed to sialoglycoconjugates of human erythrocytes. Sialylated sequences on erythrocyte membranes serve as receptors for *M. pneumoniae* adhesion [57]. It is hypothesized that the binding of *M. pneumoniae* to the receptors renders them autoimmunogenic, inducing cold agglutinin production [58].

Mycoplasma-induced arthritis in animals has been repeatedly advocated as a convenient model for studying human rheumatoid arthritis and as-
sociated ocular inflammation. The rodent model of \textit{M. arthritidis}-induced polyarthritis permits easy access to the multiple events that result in experimental arthritis, including ocular inflammation. Following the acute phase of arthritis, mice develop a chronic proliferative polyarthritis that exhibits periods of remission and exacerbation and which closely resembles histologically the lesions seen in human rheumatoid arthritis. Antigenic mimicry, specific tissue interactions and immune complex involvement have been proposed as factors contributing to \textit{M. arthritidis} pathogenicity [59,60].

An intriguing question is whether mycoplasmas, alive, dead or products thereof, can be detected in inflamed tissue. Kirchhoff et al. [61] reported the rapid clearing from the blood of \textit{M. arthritidis} inoculated via the rat tail vein. Only dead or fragmented mycoplasmas trapped with related antibodies were detectable in serum samples. However, although no longer present in the blood, viable mycoplasmas have been isolated from a variety of organs, with joint isolates evident up to 200 days following infection. Search for viable mycoplasmas in joints of rheumatoid arthritis patients has failed so far. Yet, it may be worthwhile to look for mycoplasma cell components, such as RNA and DNA, using the highly sensitive DNA probes and PCR.

\textit{M. arthritidis} produces a potent mitogen (a 27-kDa protein designated MAM) recently defined as a superantigen [62]. Superantigens, produced by diverse microbial agents, activate T cells by a unique pathway which can lead to modification of the T cell repertoire and induce autoimmunity. Cole and Atkin [62] propose that MAM participates in the chronic joint inflammation in rats and mice infected by \textit{M. arthritidis}, not only by activating T cells with a resulting liberation of inflammatory lymphokines, but also by suppressing host defenses. The role of infection versus autoimmunity in the chronic phase of the disease remains to be established. The \textit{M. arthritidis}-induced arthritis may prove to be an ideal model for studying a chronic disease since it is produced by an organism that generates a potent superantigen. An attractive hypothesis is that the episodic nature of the disease, as well as that of the human rheumatic diseases, might be due to a subsequent re-infection with superantigen-producing organisms.

Chronicity of mycoplasmal infections raises an interesting question: how do the wall-less, rather fragile, mycoplasmas resist the immune defense mechanisms of their host? It now appears that at least some of the pathogenic mycoplasmas undergo high-frequency phenotypic switching phenomena, involving variable antigens, and in this way evade host defenses [63–70]. Perhaps the best characterized of these variable antigenic systems is that described for \textit{M. hyorhinis} by Wise and his colleagues. Antigenic variation in this swine pathogen is generated by combinatorial expression and phase variation of multiple abundant surface acylated lipoproteins (VLps). These products also vary markedly in size through spontaneous changes in periodic protein structures [67,69,70]. The genetic basis for this size and antigenic variation has recently been elegantly elucidated by Yogev et al. [71] showing that the system is governed by a cluster of three related, but divergent vlps genes, which encode conserved N-terminal domains for membrane insertion and lipoprotein processing, but divergent external domains undergoing size variation by loss or gain of repetitive intragenic coding sequences. Spontaneous mutation within 5′ regulatory sequences of vlps genes is implicated as an element controlling VLP phase variation [71].

5. MYCOPLASMAS AND AIDS

A most intriguing as well as controversial subject has been raised by Shyh-Ching Lo of the Armed Forces Institute of Pathology in Washington. In his first publication in 1986 Lo claimed to have isolated a novel virus from Kaposi sarcoma cells and other tissues from AIDS patients [72,73]. As this agent was later shown to carry ribosomal genes, its definition was amended to a ‘virus-like infectious agent’ [74]. The successful cultivation of the ‘virus-like agent’ in cell-free medium led to its identification as a novel pathogenic mycoplasma named by Lo et al. [54] \textit{Mycoplasma incognitus}. However, this definition proved also
to be taxonomically wrong, as genetic and serological testing indicated that the ‘new’ mycoplasma is a strain of *Mycoplasma fermentans*, a well-established mycoplasma species [75]. *M. fermentans*, first isolated from the genital tract of apparently healthy individuals in 1950, has long been suspected to be associated with human disease. Thus, in the 1960s, *M. fermentans* strains were isolated from the bone marrow of leukemia patients [76] and were also incriminated as causative agents of rheumatoid arthritis [77]. None of these claims, however, has gained enough support to endow *M. fermentans* with the status of a human pathogen. Recent studies of Muhlradt et al. [78,79] revive the possibility that *M. fermentans* has a role in rheumatic disease in humans.

The fact that the early work by Lo et al. [72] was based on detection of the new agent in cell cultures inoculated with tissues from AIDS patients, and its final identification as a strain of *M. fermentans*, a known cell culture contaminant [80] of dubious pathogenic potential, has been taken by the sceptics as evidence against any role this mycoplasma may play in AIDS. However, the detection of *M. fermentans* strain incognitus in tissues, blood and urine of AIDS patients, employing electron microscopy, in situ labelling with specific antibodies or with DNA probes, or by using PCR with specific *M. fermentans* genomic sequences as primers [54,81–83] make Lo’s observations and interpretations difficult to dismiss as artefacts, even if his original mycoplasma isolate was indeed a cell culture contaminant. Moreover, *M. fermentans* strain incognitus was not only identified in tissues of immunocompromised AIDS patients, but was shown [84] to cause a fulminant flu-like fatal infection in six non-AIDS patients, a finding supported by a more recent report of a similar case responding well to tetracycline treatment [85]. Nevertheless, as long as the in situ and DNA probe demonstrations of *M. fermentans* in patients are not supported by culture of the organism, doubts as to its pathogenic role will remain with us. Efforts of cultivating *M. fermentans* directly from patients with AIDS on acute respiratory disease have, therefore, continued, [81] but so far with no conclusive published results. In light of the significant genotypic heterogeneity characterizing many mycoplasma species [10,17] it is possible that the *incognitus* strain differs from other *M. fermentans* strains in its pathogenic potential. Comparative analyses of the ‘incognitus’ with other *M. fermentans* strains [75,86] have, however, failed to provide a clear answer to the above suggestion.

AIDS is characterized by a profound alteration in the function and number of T4 lymphocytes, resulting directly or indirectly from infection with HIV-1 or HIV-2. Several hypotheses were proposed to explain the marked cell killing, but none of them is sufficient to completely explain the extensive in vivo T4 cell destruction. Luc Montagnier has, therefore, entertained the notion that a microorganism can play the role of a cofactor in HIV-induced cell lysis. The early study by Lemaitre et al. [87] showing the inhibition of HIV-induced cytopathic effects in cell lines by tetracycline analogs, to which mycoplasmas are sensitive, was recently confirmed and extended with other antimycoplasmal agents [88]. HIV replication was not affected by the antibacterial agents. Lo et al. [89] basically confirmed the observations of Montagnier’s group by showing that co-infection of T lymphocyte cell cultures with *M. fermentans* (strain incognitus) enhances the ability of HIV-1 to induce cytopathic effects without affecting virus replication. While no clear explanation is available for the mechanism of the synergistic in vitro effects of HIV-1 and mycoplasmas, these observations inevitably lead to the suggestion that this in vitro synergism may also exist in vivo and thus have clinical implications. It should be brought up at this point that many mycoplasmas have been shown to share a complex relationship with the immune system. Stimulatory as well as suppressive effects have been described [90]. The recent studies by Muhlradt et al. [78,79] show that a high-molecular mass component of *M. fermentans* cells generates cytolytic T cells from mitogen-stimulated murine thymocytes, by a process involving stimulation of IL-1 and IL-6 synthesis by adherent accessory cells. These monokines serve in turn as mediators for IL-2 and IL-4 synthesis. Although Muhlradt et al. [78,79] have been interested in the possible role of *M. fermentans* in rheumatic
disease in humans, these observations may well be relevant to the understanding of the possible role of *M. fermentans* as a pathogen and a cofactor in AIDS.

In conclusion, it appears that a citation from the Lancet Editorial (5 January, 1991) is still valid: “Could it be that the mycoplasmas behave as opportunistic organisms, cofactors in the pathogenesis of AIDS, or pathogenic agents on their own right? None of these possibilities can be excluded, but fascination about the subject remains linked with scepticism”. One thing is for sure: the interest in mycoplasmas generated through the AIDS issue has already proved to be productive, leading to the discovery of a new mycoplasma, *M. penetrans* [52,53], and to the realization that mycoplasmas are not only ‘surface parasites’ but can penetrate, sometimes actively, into animal tissues and cells. Another lesson to be learned is to keep an open mind to the possibility that there are more human mycoplasmas not described so far. Thus, we should not discount easily any claim, such as that by Wirsotko et al. [91] for the existence of human pathogenic mycoplasmas, uncultured and unidentified thus far.

REFERENCES


