Mycoplasmas and Nucleases

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(Received: 20 April 2015; accepted: 02 June 2015)

Mycoplasmas cell wall-less bacteria of the class mollicutes, are among the smallest self-replicating organisms known and have been described as minimal living units. The presence of a small genome and the lack of numerous biosynthetic pathways correlate with growth requirements for macromolecular precursors such as phospholipids, cholesterol, amino acids, and nucleotides. Mycoplasma nuclease are thought to play a metabolic role in the production of nucleotide substrates from host or microbial nucleic acids released through natural and induced cell death. Nuclease activity in members of the mollicutes has been proposed as the mechanism by which these organisms acquire the precursors required for their nucleic acids. Nucleases are multifunctional enzymes and widespread in distribution. Intracellularly, they have been implicated in recombination, repair and replication, whereas extracellular enzymes have a role in nutrition. Nucleases are thought to be important contributors to virulence and crucial for the maintenance of a nutritional supply of nucleotides in mycoplasmas that are pathogenic in animals and human. Nucleases detection is a significant findings for an etiologic diagnosis and for determining the virulence of the mycoplasma strain isolated in the laboratory. Because of the importance involving nucleases, presented this review.

Key words: Nuclease, mycoplasma, nutrition source, nucleic acids.

Mycoplasmas represent the smallest self-replicating organisms, in the both cellular dimensions and genome size that are capable of cell-free existence. The small size and volume of mycoplasmal cells allow them to pass through 0.45 µm pore size filters that are commonly used to filter sterilize media. The small cellular mass also means that mycoplasmas cannot be detected by light microscopy, and they do not produce visible turbidity in liquid growth media.

Mycoplasmas are widespread in nature as parasite of plants, arthropods, fish, reptiles, mammals, and humans. The primary habitats of human are the mucous surfaces of the respiratory and urogenital tracts, the eyes, alimentary canal, mammary glands, and joints1,2.

Infections with pathogenic mycoplasmas are rarely of the fulminant type but, rather, follow a chronic course. A review referring also to the variety of diseases of unknown etiology that have been linked to mycoplasmas3.

Mycoplasmas may be the only procaryotes which can symbiotically grow in the eucaryotic host and have a close interaction with mammalian cells for long periods. Intimate interactions of the mycoplasmas with the host cell surface may trigger a cascade to signals transduced from the cell membranes to the nuclei, altering the function of many genes4.

It has been proposed that the intimate contact of the wall-less mycoplasmas with the host cell membranes or exchange of membrane components and hence in direct injection of the mycoplasma cytoplasmic content, including hydrolytic enzymes and nucleases combined with superoxide radicals may be responsible for
clastogenic effects. Because of the importance involving nucleases, present this review.

**Nucleases features**

Nucleases catalyzing DNA and RNA cleavage are indispensable for life, nuclease activities are integral parts of DNA replication; the 5´ to 3´ exo- and endonucleases are needed to remove RNA primers, and the 3´ to 5´ exonuclease for proofreading. Two other major DNA metabolic processes, recombination and repair, are initiated by nucleases. Nuclease activity is also required for structural alterations of nucleic acids, for example, topoisomerization, site-specific recombination and RNA splicing during which a phosphodiester bond is temporarily broken and reformed after strand passing or transfer to a new target. In addition, nuclease activities are essential in RNA processing maturation and RNA interference. RNA and DNA degradation are essential components of microbial defense mechanisms, nucleases are even essential for programmed cell death. Defective DNase and RNase activities have been associated with various autoimmune diseases due to an incomplete removal of endogenously produced nucleic acids.

**RNA and DNA present only two types of phophodiester bonds for cleavage, 5´ or 3´ of a scissile phosphate, and the fundamental chemistry is bimolecular nucleophilic substitution or SN2 in short. Nonetheless, structure and catalytic mechanisms of RNA and DNA nucleases are greatly varied and complex. Nucleases can be protein or RNA and use water, deoxyribose, inorganic phosphate, or the side chain of Ser, Tyr or His as a nucleophile. Nucleases activities are strictly regulated by stringent substrate specificity, confined localization, or by potent inhibitors to avoid unwanted or uncontrolled degradation of cellular DNA and RNA.

The Serratia family of nucleases includes bacteria nucleases (NucA) and eukaryotic mitochondrial endonuclease G (Endo G), which is also known as Nuc1p in yeast. The nuclease from *Serratia marcensces* encoded by the *nucA* gene is the first example described in the family. Its possible role is scavenging for nutrients, other bacterial NucA may act as virulence factors in invasion or establishment of a colony. Bacterial host, which secrete the nuclease, often have an inhibitor to protect themselves. In eukaryotes, mitochondrial Endo G may play a role in mitochondrial DNA metabolism. Endo G, however, can migrate to the nucleus and function in Caspase-independent cell death. These nucleases are structural very similar, the catalytic bba motif forms a structural subdomain and is packed against one face of a six-stranded antiparallel b-sheet. The active site is marked by a conserved sequence motif RG immediately preceding the general bases H in the first b-strand of the bba motif. They usually prefer Mg$_2^+$ for catalysis, and the metal ion is coordinated by the conserved N and E on the a-helix. The catalytic metal ion may be directly chelated by an immunity protein and prevented from substrate binding.

Nuclease P1 and S1 are single-strand-specific nucleases that degrade DNA or RNA either endonucleolytically or as a 3k exonuclease. P1 nuclease is all a-helical and resembles bacterial phospholipase C. Although P1 nuclease and Endo IV are unrelated in amino acid sequence or tertiary structure, they both use carboxylates and histidines to tightly bind three Zn$_{3+}$ ions even in the absence of substrate. In P1 nuclease, two Zn$_{3+}$ ions are jointly coordinated by two conserved Asp. Similar to Endo IV, the pro-Rp rather than the pro-Sp non-bridging oxygen of the scissile phosphate is intolerant of thio-replacement. Although a P1-substrate complex structure is not available, the sulfur replacement experiment is in agreement with the metal ion coordination observed in Endo IV. It is likely that the pro-Rp oxygen of the scissile phosphate together with the two conserved Asp residues coordinates the two catalytic metal ions.

DNase I is most prevalent in mammals and is also found in bacteria, but not in low eukaryotes or plants. DNase I functions in apoptosis and has been implicated in the autoimmune disease systemic lupus erythematosus. The crystal structure off bovine pancreatic DNase I-DNA complex was determined nearly 20 years ago. In the crystal structures of DNase I without DNA, two Ca$_{3+}$ ions were observed at a distance from the active site and thought to play structural and substrate-binding roles. DNase I is most active in the presence of mixed Ca$_{3+}$ and Mg$_{2+}$, all the catalytic residues (four Asp and Glu, two His and one Asn) are located at the end of the central 4 b-strand of both b-sheets. Two conserved His
residues surrounding the scissile phosphate haven shown to be important for the general acid-and-base catalysis, and the His mutations can be rescued by imidazole. The scissile phosphate is distorted and both pro-Rp and pro-Spoxysgens interact with DNase I. Although divalent cation is chelated away by 20 mM EDTA and thus absent in the crystal structure of enzyme-substrate complexes, the requirement of two Asp residues and Mg$_2^+$ for DNA cleavage suggest that metal ions are likely to be essential for catalysis$^{21-23}$.

Classification of nucleases and their function is presented: (1) DNA replication (function: proofreading, primer removal), (2) Rolling-circle replication (function: nicking), (3) DNA replication, transcription, recombination (function: topoisomerization), (4) Site-specific recombination (function: retroviral integration and recombination transposition, gene inversion, phage integration and plasmid segregation), (5) DNA homologous recombination (function: processing broken DNA ends HJ resolution), (6) DNA repair (function: meiosis breaks, incision in base excision repair, incision in nucleotide excision repair, incision and removal in mismatch repair, double-strand break repair), (7) RNA processing (function: tRNA 5' processing, tRNA 3' processing, tRNA splicing, mRNA splicing, mRNA end processing and degradation), (8) RNA interference (function: dicer, slicer), (9) Cell death (function: DNA decay, RNA decay), (10) Defense (function: restriction/modification, microbial toxins), and (11) Nutrition source (function: DNA digestion and scavenging, RNA digestion)$^{24}$.

Catalytically important amino acid residues of nuclease have been identified by structural analysis, sequence comparison, and by site-directed mutagenesis. Mutations of residues R57, R87, H89, N119 and E127 resulted in enzymes that were found to be catalytically inactive confirming that these residues constitute the active site of nuclease$^{25-27}$.

In view of their prominent location in the center of the active site of the nuclease model, the amino acid residues H89 and E127 were primary targets for mutational and biochemical investigations. One proposed reaction mechanism predicts that H89 acts like a general base (Figure 1), which abstracts a proton from a water molecule, activating it for a nucleophilic attack on the phosphorus atom adjacent to the scissile bond. Interestingly a recent structure determination of nuclease identified N119 as being bound to Mg$_2^+$, therefore the stabilizing role of N119 is likely mediated by this Mg$_2^+$. This residue could have an additional role in positioning the attacking water molecule relative to the phosphorus atom. A similar function has been discussed for R57, acting through its guanidinium group$^{28}$.

An alternative model suggesting that H89 might instead function as the general acid, protonating the leaving group, and E127 being the general base (Figure 2) has also been proposed. However the ability of H89A to cleave the artificial chromophoric substrate deoxythymidine 3',5'-bis(p-nitrophenyl-phosphate), which does not require protonation of the leaving group, appears to contradict this model. Mutant H89A is inactive with this nucleotide analogue, whereas an E127A mutant still cleaves. This argues that E127 is dispensable for the initial water activation, and therefore by default H89 is the opposite, at least for this artificial substrate$^{28-30}$.

When the role of those amino acid residues that are essential for nuclease activity, but are not directly involved in the catalysis reaction, was studied by site-directed mutagenesis, three additional residues outside of the active center were identified. R87, R131 and possibly also D86 mutants are mainly affected in their ability to bind nucleic acid substrate but not in their catalytic activity. More specifically, R87 is thought to interact with the phosphate group at the 3P-end of the ribose sugar, which is essential for cleavage near the 5'-end. With regard to the nuclease model, all three residues are located in the putative substrate binding site of the enzyme, suitably positioned to assist in positioning diverse nucleotide substrates into a conformation that is accepted by the enzyme$^{26}$.

Nuclease expression

Nuclease production is regulated and the parameters of its regulation are not initially obvious. Nuclease expression is not substrate regulated, the addition of nucleic acid does not induce its expression nor does the addition of free nucleotides repress it. It also is not catabolite regulated, instead environmental signals control nuclease expression. Transcription increases as growing cultures increase in density and approach...
saturation. Research suggests this is modulated by a factor released by the bacteria into the growth media, likely to be related to the HSL signaling molecules31, 32.

A transcriptional regulator which acts at the mucA promoter as an activator of transcription is the NucC protein. This protein is a member of the P2 Org family of phage transcriptional activators and likely interacts with the α subunit of RNA polymerase. NucC binds to a region between -82 and -51 upstream of the transcriptional start of mucA as determined by footprinting analysis and this region includes a copy of the TGT-N12-ACA activator recognition motif. Transcription of mucC appears also to be growth phase regulated. The simplest model would have extracellular density signals modulate production of NucC which in turn activates mucA transcription33.

nucC lies in an operon with two other genes, nucD and nucE, these bear a strong resemblance to phage proteins, specifically NucE resembles holing proteins involved in releasing lysozyme to the peptidoglycan of Gram-negative bacteria and NucD is such a lysozyme. These proteins appear to play no significant role in nuclease secretion; their deletion from the Serratia genome does not affect nuclease secretion. A second environmental signal known to regulate nuclease expression is the bacterial SOS system. Nuclease production is increased strongly by agents which induce SOS controlled genes34, 35.

Nuclease role in mycoplasmas

Mycoplasma nuclease was first reported by Razin et al., (1978) these enzymes have been suggested to be involved in DNA. In addition, several authors have reported that the nucleic acids of host cells may be targets for soluble nuclease secreted into the extracellular medium and/or bound to mycoplasma membranes. Indeed, these bacteria are deficient in nucleotide biosynthesis pathways. Their nuclease activities, on the other hand, are developed as a means of producing the nucleic acid precursors required for their metabolism by digesting the DNA and RNA of the cells they parasitize36-39. As a consequence have shown that nucleases produced by contaminating mycoplasmas were responsible for the apparent absence of reverse transcriptase activity in the supernatants from HIV-producing cell lines40, 41.

These nuclease activities of mycoplasmas were recently implicated in the induction of apoptosis characterized by the internucleosomal fragmentation of the chromatin of infected cells42-44.

Mycoplasmas are generally defective in several metabolic pathways and their growth requires macromolecular precursors including nucleic acids from the host and/or the surrounding medium. They cannot synthesize purine and pyrimidine bases as a mechanism enabling them to acquire nucleic acids in the form of free bases and/or oligonucleotides. The capacity of some species, including M. penetrans, to invade the cells they parasitize suggests that host cell DNA and/or RNA could be a substrate for these nuclease activities38, 39.

Cells incubated with the endonuclease exhibited considerable cytopathic effects. The alterations included condensation of the cytoplasm, a loss of surface microvillousness, and the appearance of apoptotic bodies, consistent with the reduction in cell numbers. The nucleic acids of cells incubated with endonuclease P40 were analyzed by agarose gel electrophoresis and showed an oligonucleosomal fragmentation of chromatin similar to that observed in apoptotic cells45.

The cytotoxic effects of endonuclease P40 toward lymphocytes in vitro that may be susceptible to infection in vivo by M. penetrans. This microorganism can exert two simultaneous effects via P40: a cytotoxic effect caused by P40 secreted by extracellular M. penetrans, and a second effect caused by P40 secreted by M. penetrans inside the cells. The latter form of endonuclease P40 could rapidly and directly be at the origin of chromatin degradation that causes cell death. Endonuclease P40 as a potential virulence factor in infection caused by this mycoplasma45. These data suggest that in a large majority of mycoplasmal infections, parasite nuclease activities can participate directly in cell death by apoptosis, in addition to the mechanisms inducing cellular necrosis. Endonuclease activity can be implicated, at least in vitro, as a potential factor in the degradation of the nucleic acids of parasitized cells suggests a potential role of mycoplasmal nuclease as pathogenic factors.

Most mycoplasmas cannot synthesize any fatty acid and therefore depend on the host
for their supply. Although mycoplasmas generally synthesize their own membrane phospholipids and glycolipids from the exogenously provided fatty acids, some mycoplasmas incorporate preformed host phospholipids into their membrane.

Studies on mycoplasma nutrition revealed the requirement for the nucleic acid precursors, purines and pyrimidines. These may be provided by RNA and DNA that have been degraded by the potent mycoplasmal nuclease.

Chronic infection or colonization by mycoplasmas could gradually and significantly alter many biologic properties of mammalian host cells in culture, including induction of malignant transformation. Compared with spontaneously immortalized peripheral blood mononuclear cells (PBMCs), the PBMCs immortalized in cultures infected with the mycoplasmas often had prominent karyotype changes with chromosomal loss, gain, or translocations. Mycoplasmas can grow in close interaction with mammalian cells, often silently for a long period of time. However, prolonged interactions with mycoplasmas with seemingly low virulence could, through a gradual and progressive course, induce chromosomal instability as well as malignant transformation, promoting tumorous growth of mammalian cells.

M. fermentans mediated transformation of cells has long latency and demonstrates distinct multistage progression.

The infection of the 32D cell line with M. hyorhinis and M. fermentans induced compression of the nucleus, degradation of cell genome and dysregulation of the expression of genes related to proliferation, apoptosis, tumorigenesis, signaling pathway and metabolism.

Mycoplasmas, cell wall-less bacteria of the class Mollicutes, are among the smallest self-replicating organisms known and have been described as minimal living units. The presence of a small genome and the lack of numerous biosynthetic pathways correlate with growth requirements for macromolecular precursors such as phospholipids, cholesterol, nucleotides, and amino acids. Nuclease activity in members of the Mollicutes has been proposed as the mechanism by which these organisms acquire the precursors required for their nucleic acids.

These presence of nucleases in mycoplasmas has been reported, nucleic acids precursors are growth factors of mycoplasmas. This growth requirement can be met by nucleosides or undegraded DNA or RNA. Karyological and morphological changes in human diploid cells infected with Mycoplasma pulmonis and associated these changes with competitive mycoplasmal interference of host DNA synthesis. Human pathogen Mycoplasma pneumoniae in tracheal explants induces ciliostasis and decreases cellular respiration, dehydrogenase activity, rate of oxygen uptake, and ATP content and have related all of these aberrations to ATP and adenine levels. Infection with Mycoplasma pneumoniae causes a diminution of host protein and RNA synthesis. The nuclease of parasitic mycoplasmas may contribute to host pathology by catalyzing reactions which produce mycoplasmas nucleic acid growth factors from host nucleic acid precursors or DNA.

Mycoplasma nucleases are thought to play a metabolic role in the production of nucleotide substrates from host or microbial nucleic acids release through natural and induced cell death. These nucleotide substrates may be produced following the important of small oligonucleotides into the cytoplasm or through the extracellular activity of nucleases attached to or secreted from the cell surface. Membrane-associated nuclease activity has been identified in all mycoplasma species studied so far. The identification of intracellular, extracellular, and membrane-associated nuclease activities in a number of mycoplasma species suggest the involvement of nucleases in a variety of cellular processes.

During the last five years, several studies highlight the importance of studying in mollicutes nucleases, highlighting mycoplasma species of medical importance, such as, Mycoplasma pneumoniae, Mycoplasma genitalium and Mycoplasma fermentans.

Nuclease has also been shown to have significant anti-tumor properties, presumably by interfering with replication of dividing cells. Obviously a suitable targeted delivery system is the limiting factor for an effective treatment, something also lacking for many other molecules with similar antitumor properties. Of significant importance to the use of nuclease in any therapeutic regime is its ability to be tolerated by the mammalian immune system.
### Table 1. Proteins nuclease activity described in mycoplasmas species.

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All living systems contain nucleases, capable of interacting with nucleic acids and hydrolyzing the phosphodiester linkages. The enzymatic breakdown of nucleic acids was first observed in the early twentieth century, and the term ‘nucleases’ was coined for enzymes involved in this. However, it was not until 1940 that Kunitz described two groups of nucleases based on sugar specificity, and subsequently different schemes of classification were proposed. With the discovery of newer nucleases and multifunctional enzymes like micrococcal nuclease and snake-venom phosphodiesterase, however, the classification of Kunitz was found to be inadequate. Soon, a new class of sugar non-specific nucleases had to be added to the list as per new evidence. Hence, to overcome these shortcomings, Bernard and Laskowski suggested that nucleases be classified on the basis of: (1) the nature of substrate hydrolyzed (DNA, RNA); (2) the type of nucleolytic attack (exonuclease and/or endonuclease); (3) the nature of the hydrolytic products formed i.e. monoo or oligonucleotides terminating in a 3’- or a 5’-phosphate; and (4) the nature of the bond hydrolyzed.

The pathogenesis of most mycoplasmas is predominantly attributable to the immunopathological response of the host to the persistent presence of these pathogens. Therefore, virulence genes in mycoplasmas are probably best defined as those that are not necessary for growth in vitro, but that are required for optimal colonization of, persistence in our pathological effects on the host, including those genes whose primary function appears to be ensuring optimal nutrient acquisition in vivo. In addition it has...
become clear that several systems for optimal pathogenicity, in some cases because these nutrients contribute to the generation of toxic metabolites, while in others presumably because they facilitate persistence in a nutrient limited environment.

A number of mycoplasma proteins, particularly those exposed on the cell surface, have been shown to have multiple functions. This is perhaps not surprising in organisms with a minimal genome, as the use a single protein to fulfill multiple roles is likely to contribute significant genomic economy. The complex nutritional requirements of mycoplasmas when cultured in vitro, and their relative dearth of biosynthetic capacity, suggest that they require efficient mechanisms for acquiring complex nutrients from their surroundings. These mechanisms are likely to include an ability to degrade macromolecules into importable subunits, as well as efficient binding protein and transport mechanisms.

Reports on chromosomal aberrations, altered morphology, and cell transformation in cell cultures infected by mycoplasmas have appeared rather sporadically since the early 1960s. Interest in this subject has been recently rekindled following the claims that *M. penetrans* may be a cofactor in the induction of HIV-associated Kaposi’s sarcoma.

Another issue that came up recently concerns the possible role of mycoplasma infection on the induction of apoptosis. DNA fragmentation, a common biochemical hallmark of apoptosis, is generally considered to be catalyzed by endogenous endonucleases. The infected cells exhibited intranucleosomal DNA degradation into multimers of 200 bp, forming a ladder in agarose gels. Nuclease activities were detected in cell homogenates and culture supernants.

The mycoplasma membrane is rich in essential enzymes, and most the nuclease activity of all mycoplasma species studied so far is either located at or all secreted from the cell surface. The identification of an amino-terminal signal sequence and prokaryotic lipoprotein cleavage site indicates that mhp379 was examined by using antiserum raised against recombinant mhp379 in western immunoblots of TX-114-fractionated proteins and trypsin-treated *M. hyopneumoniae* cells. These results indicate that mhp379 is a 33-kDa membrane-associated protein exposed on the cell surface.

CONCLUSION

Mycoplasmas can grow in close interaction with mammalian cells, often silently for a long period of time. However, prolonged interactions with mycoplasmas with seemingly low virulence could, through a gradual and progressive course, induce chromosomal instability as well as malignant transformation, promoting tumorous growth of mammalian cells. This mycoplasma mediated transformation of cells has a long latency and demonstrates distinct multistage progression. Nucleic acids precursors are essential for the growth of all mycoplasmas and the acquisition of nucleic acid precursors is thought to involve the degradation of exogenous nucleic acids by mycoplasma nucleases located at or secreted from the cell surface. This is supported by the identification of transport mechanisms for the import of exogenous nucleotides and the identification of membrane-associated nuclease activity in all mycoplasmas species studied so far.
REFERENCES


