JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2015. Vol. **9**(3), p. 2115-2125

Mycoplasmas and Nucleases

A. Rivera* and L. Cedillo

Laboratorio de Micoplasmas del Centro de Investigaciones en Ciencias Microbiologicas, Instituto de Ciencias de la Universidad Autonoma de Puebla. Mexico.

(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 joints[1-2].

* To whom all correspondence should be addressed.
E-mail: jart70@yahoo.com

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 mycoplasmas[3].

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 genes[4].

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[5]. 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[6]. 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 bonds 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[7-10].

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 S_N2 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[11].

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[12-14].

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_2+ ions even in the absence of substrate. In P1 nuclease, two Zn_2+ 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[15-17].

DNase I is most prevalent in mammals and is also found in bacteria, but not in low eukaryotes or plants. DNase I functions in apopotosis 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_2+ ions were observed at a distance from the active site and thought to play structural and substrate-binding roles[18-20]. DNase I is most active in the presence of mixed Ca_2+ 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-Spoxygens 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: topoisomerizacion), (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 suggest this is modulated by a factor released by the bacteria into the growth media, likely to be related to the HSL signaling molecules[31, 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± subunit of RNA polymerase. NuC 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 *nucA* transcription[33].

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 genes[34, 35].

Nuclease role in mycoplasmas

Mycoplasma nucleases were 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 nucleases 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 nuclei acid precursors required for their metabolism by digesting the DNA and RNA of the cells they parasitize[36-39]. 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 lines[40, 41].

These nuclease activities of mycoplasmas were recently implicated in the induction of apoptosis characterized by the internucleosomal fragmentation of the chromatin of infected cells[42-44].

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 synthetize 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 activities [38, 39].

Cells incubated with the endonuclease exhibited considerable cytopathic effects. The alterations included condensation of the cytoplasm, a loss of surface microvillosities, 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 cells[45].

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 mycoplasma[45]. 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 nucleases 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[38].

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 nucleases[36].

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[46].

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[47].

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[36].

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[48].

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 specie suggest the involvement of nucleases in a variety of cellular processes[49, 50].

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*[51-57].

Nuclease has also been shown to have significantanti-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[58-60].

Table 1. Proteins nuclease activity described in mycoplasmas species.

Clade ID	Organism	Proteinname	Accession	Locus_tag	Length (aa)	Identicalgroup
21069	*Mycoplasma canis* UF33	Nuclease	WP_004796101	MCANUF33_00395	411	WP_004796101
21069	*Mycoplasma canis* UFG1	Nuclease	WP_004796579	MCANUFG1_00395	411	WP_004796579
21069	*Mycoplasma canis* UFG4	Nuclease	WP_004796967	MCANUFG4_00274	411	WP_004796967
21069	*Mycoplasma canis* PG14	Endonuclease	WP_004794501	MCANPG14_01013	223	WP_004794501
21069	*Mycoplasma canis* UF31	Endonuclease	WP_004795669	MCANUF31_00983	194	WP_004795669
21069	*Mycoplasma canis* UF33	Endonuclease	WP_004796193	MCANUF33_01008	196	WP_004796193
21069	*Mycoplasma canis* UFG1	Endonuclease	WP_004796643	MCANUFG1_00968	195	WP_004796643
21069	*Mycoplasma canis* UFG4	Endonuclease	WP_004797052	MCANUFG4_00988	195	WP_004797052
22303	*Mycoplasma cynos* C142	Putative DNA/RNA nuclease	WP_015287562	MCYN_0708	192	WP_015287562
22296	*Mycoplasma flocculare* 27716	Hypotheticalprotein	WP_002557560	MFC_00624	112	WP_002557560
21059	*Mycoplasma hyopneumoniae* 168-L	Nuclease	WP_014579729	MHP168L_291	112	WP_014579729
21059	*Mycoplasma hyopneumoniae* 232	Nuclease	WP_011205947	mhp109	112	WP_011205947
21065	*Mycoplasma fermentans* M64	Hypotheticalprotein	WP_013526821	MfeM64YM_0384	54	WP_013526821
21065	*Mycoplasma fermentans* M64	Nuclease PIN	WP_013526869	MfeM64YM_0455	79	WP_013526869
21065	*Mycoplasma fermentans* PG18	Nuclease PIN	WP_013526823	MBIO_0116	62	WP_013526823
21065	*Mycoplasma fermentans* PG18	Hypotheticalprotein	WP_015511040	MBIO_0557	83	WP_015511040
21061	*Mycoplasma hyorhinis* GDL-1	Nuclease	WP_014335566	MYM_0384	434	WP_014335566
21066	*Mycoplasma agalactiae*	Nuclease	WP_013021746	MAGa0859	190	WP_013021746
22820	*Mycoplasma bovis* PG45	Nuclease	WP_013456560	MBOVPG45_0089	190	WP_013456560
22296	*Mycoplasma flocculare* ATCC 27716	Membrane nuclease, lipoprotein	WP_002557711	MFC_00420	377	WP_002557711
21058	*Mycoplasma ovipneumoniae* SC01	Nuclease	WP_010321483	MoviS_0101100003608	395	WP_010321483
21051	*Mycoplasma gallisepticum* S6	Membrane Nuclease MnuA	WP_011883622	GCW_00815	389	WP_011883622
21051	*Mycoplasma gallisepticum* S6	Nuclease-likelipoproteina	WP_011883659	GCW_00940	276	WP_011883659
21051	*Mycoplasma gallisepticum* NC06_2006.080-5-2P	Nuclease	WP_014886513	HFMG06NCA_0074	389	WP_014886513

http://www.ncbi.nml.nih.gov/proteinclusters/

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. monoor oligonucleotides terminating in a 3´- or a 5´-phosphate; and (4) the nature of the bond hydrolyzed[61].

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

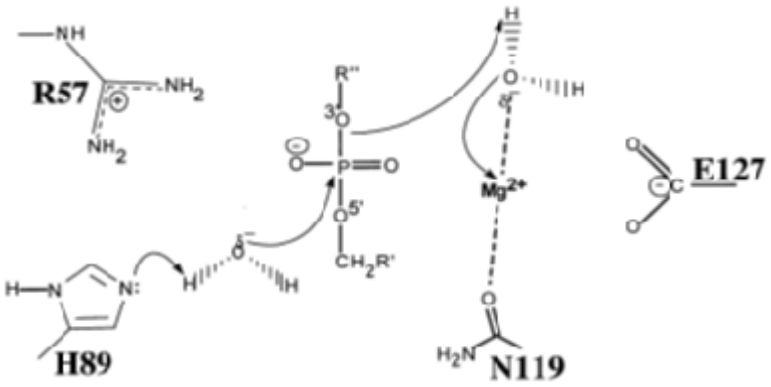

Fig. 1. Model of the catalytic mechanism nuclease attack, H89 in the active center of the enzyme is perceived to act either as a general base.

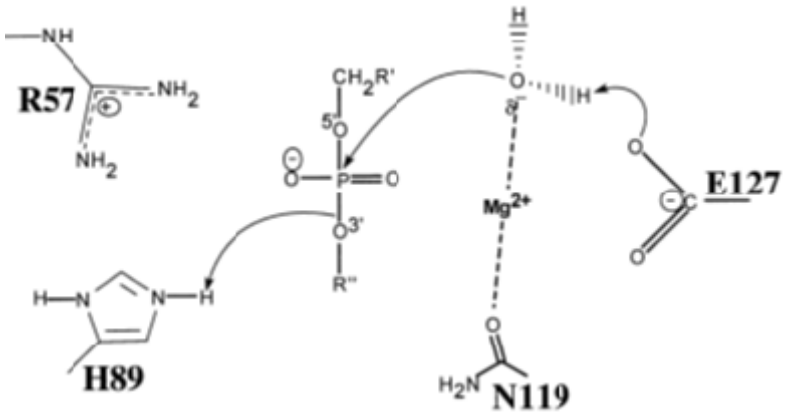

Fig. 2. Model of the catalytic mechanism nuclease attack, a general acidin a Mg^{2+}dependent hydrolysis reaction.

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[62].

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[63].

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[64, 65].

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[43].

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. Genetic analysis in mycoplasmas has revealed the presence of proteins with characteristics of nucleases, here are summary of the protein have been described, highlighting the species, protein name and length (Table 1). The nuclease activity of most mycoplasma species appears to be largely associated with the cell membrane. No transport mechanism for the import of oligonucleotides has yet been identified, and nucleases located in the cytoplasm require stringent control of activity. Nucleases located in the cytoplasm are thought to be primarily involved in recombination and repair and in restricting foreign nucleic acids. The import of exogenous nucleotides usually precedes their dephosphorylation, and a membrane-associated protein with similarity to bacterial 5´ nucleotidase was recently show to be expressed by *M. hyopneumoniae* in infect pigs. However, *M. mycoides*subspecie*mycoides* has a novel ability to import and utilize nucleotide5´ monophosphates without prior dephosphorylation[66-68]. Nucleases are involved in a variety of cellular processes, and genomic sequence analyses have identified multiple genes encoding putative nucleases in all mycoplasma species studied so far.

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

1. Waites, K.B., Talkington, D.F. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin.Microbiol. Rev*., 2004; **17**: 697-728.
2. Rottem, S. Interactions of mycoplasmas with host cells. *Physiol. Rev*., 2003; **83**: 417-432.
3. Baseman, J.B., Tully J.G. Mycoplasmas: sophisticated, reemerging and burdened by their notoriety. *Emerging Infect. Dis*., 1997; **3**: 21-32.
4. Tsai, S., Wear, D.J., Shin, J.W.K., Lo, S.C. Mycoplasma and oncogenesis: persistent infection and multistage malignant transformation. *Proc. Natl. Acad. Sci. USA*., 1995; **92**: 10197-10201.
5. Stewart, S.D., Watson, H.L., Cassell, G.H. Investigation of the clastogenic potential of *Ureaplasma urealyticum* on human leukocytes. *IOM Lett*., 1994; **3**: 662-663.
6. Reha-Krantz, L.J. DNA polymerase proofreading: multiple roles maintain genome stability. *BiochimicaetBiophysicaActa*., 2010; **1804**: 1049-1063.
7. Grindley, N.D., Whiteson, K.L., Rice, P.A. Mechanisms of site specific recombination. *Ann. Rev. Biochem*., 2006; **75**: 567-605.
8. Chu, C.Y., Rana, T.M. Small RNAs: regulators and guardians of the genome. *J. Cell. Physiol*., 2007; **213**: 412-419.
9. Moore, M.J., Proudfoot, N.J. Pre-mRNA processing reaches back to transcription and ahead to translation. *Cell*., 2009; **136**: 688-700.
10. Crow, Y.J., Rehwinkel, J. Aicardi-Goutieres syndrome and related phenotypes: linking nucleic acid metabolism with autoimmunity. *Hum. Mol. Gen*., **18**: R130-R136.
11. Widlak, P., Garrard, W.T. Discovery, regulation, and action of the major apoptotic nucleases DFF40/CAD and endonuclease G. *J. Cell. Biochem*., 2005; **94**:1078-1087.
12. Benedik, M.J., Strych, U. *Serratiamarcescens* and its extracellular nuclease. *FEMS Microbiol.Lett*., 1998; 165: 1-13.
13. Buttner, S., Eisenberg, T., Carmona-Gutierrez, D., Ruli, D., Knauer, H., Ruckenstuhl, C.,Sigrist, C., Wissing, S., Kollroser, M., Frohlich, K.U., Sigrist, S., Madeo, F. Endonuclease G regulates budding yeast life and death. *Mol. Cell*., 2007; **25**: 233-246.
14. Ghosh, M., Meiss, G., Pingoud, A., London, R.E., Pedersen, L.C. The nuclease a-inhibitor complex is characterized by a novel metal ion bridge. *J. Biol. Chem*., 2007; **282**: 5682-5690.
15. Desai, N.A., Shankar, V. Single-strand-specific nucleases. *FEMS Microbiol. Rev*., 2003; **26**: 457-491.
16. Hough, E., Hansen, L.K., Birknes, B., Jynge, K., Hansen, S., Hordvik, A., Little, C., Dodson, E., Derewenda, Z. High resolution (1-5-A) crystal structure of phospholipase C from *Bacillus cereus*. *Nature*., 1989; **338**: 357-360.
17. Romier, C., Dominguez, R., Lahm, A., Dahl, O., Suck, D. Recognition of single-stranded DNA by nuclease P1: high resolution cristal structures of complexes with substrate analogs. *Protein*., 1998; **32**: 414-424.
18. Martinez Valle, F., Balada, E., Ordi-Ros, J., Vilardel-Tarres, M. DNase 1 and systemic lupus erythematosus. Autoimmun. Rev., 2008; 7: 359-363.
19. Weston, S.A., Lahm, A., Suck, d. X-ray structure of the DNase I-d(GGTATACC)2 complex at 2-3-A resolution. *J. Mol. Biol*., 1992; **226**: 1237-1256.
20. Chen, W., Liao, T.H. Structure and function of bovine pancreatic deoxyribonuclease I. *Protein Pept. Lett*., 2006; **13**: 447-453.
21. Pan, C.Q., Lazarus, R.A. Ca_2+dependent activity of human DNase I and its hyperactive variants. *Protein Science*., 1999; **8**: 1780-1788.
22. Chen, W.J., Lai, P.J., Lai, Y.S., Huang, P.T., lin, C.C., Liao, T.H. Probing the catalytic mechanism of bovine pancreatic deoxyribonuclease I by chemical rescue. *Biochem.Biophiys. Res. Commun*., 2007; **352**: 689-696.
23. Jones, S.J., Worral, A.F., Connolly, b.A. Site-directed mutagenesis of the catalytic residues of bovine pancreatic deoxyribonuclease I. *J. Mol. Biol*., 1996; **264**: 1154-1163.
24. Yang, W. Nucleases: diversity of structure, function and mechanism. *Q. Rev. Biophys*., 2011; **44**: 1-93.
25. Friedhoff, P., Gimadutdinow, O., Pingoud, A. Identification of catalytically relevant amino acids of the extracellular Serratiamarcescens endonuclease by alignmentguided mutagenesis. Nucleic Acids Res., 1994; 22: 3280-3287.
26. Miller, M.D., Tanner, J., Alpaugh, M., Benedik, M.J., Krause, K.L. 2.1 Å structure of Serratia endonuclease suggests a mechanism for binding to double-stranded DNA. Nature Struct. Biol., 1994; 1: 461-468.
27. Friedhoff, P., Kolmes, B., Gimadutdinow, O., Wende, W., Krause, K.L., Pingoud, A. Analysis of the mechanism the Serratia nuclease using site-directed mutagenesis. Nucleic Acids Res., 1996; 24: 2632-2639.
28. Kolmes, B., Franke, I., Friedhoff, P., Pingoud, A. Analysis of the reaction mechanism of the non speci¢c endonuclease of Serratiamarcescens

using an artificial minimal substrate. FEBS Lett., 1996; 397: 343-346.

29. Lunin, V.Y., Levdikov, V.M., Shlyapnikov, S.V., Blabova, E.V., Lunin, V.V., Wilson, K.S. and Mikhailov, A.M. Three-dimensional structure of Serratiamarcescens nuclease at 1.7 Å resolution and mechanism of its action. FEBS Lett., 1997; 412: 217-222.
30. Antosiewicz, J., Miller, M.D., Krause, K.L., McCammon, J.A. Simulation of electrostatic and hydrodynamic properties of Serratia endonuclease. Biopolymers., 1997; 41: 443-450.
31. Chen, Y.C., Shipley, G.L., Ball, T.K., Benedik, M.J. Regulatory mutans and transcriptional control of the *Serratiamarcescens* extracellular nuclease gene. *Mol. Micorbiol*., 1992; **6**: 643-651.
32. Eberl, L., Winson, M.K., Sternberg, C., Stewart, G.S., Christiansen, G., Chhabra, S.R., Bycroft, B., Williams, P., Molin, S., Givskov, M. Involvement of N-acylL-hormoserine lactone autoinducers in controlling the multicellular behavior of *Serratialiquefaciens*. *Mol. Microbiol.* 1996; **20**: 27-136.
33. Jin, S., Chen, Y.C., Chrisitie, G.E., Benedik, M.J. Regulation of the *Serratiamarcescens* extracellular nuclease: positive control by a homologue of P2 Org encoded by a cryptic prophage. *J. Mol. Biol*., 1996; **256**: 264-278.
34. Benedik, M.J., Strych, U. Serratiamarcescens and its extracellular nuclease. *FEMS Microbiol.Lett*., 1998; **165**: 1-13.
35. Desai, N.A., Shankar, V. Single-strand-specific nuclease. *FEMS Microbiol. Rev*., 2003; **26**: 457-491.
36. Minion, F.C., Jarvill-Taylor, K.J., Billings, D.E., Tigges, E. Membrane-associated nuclease activities in mycoplasmas. *J. Bacteriol*., 1993; **175**: 7842-7847.
37. Minion, F.C., Goguen, J.D. Identification and preliminary characterization of external membrane-bound nuclease activities in *Mycoplasma pulmonis*. *Infect. Immun*., 1986; **51**: 352-354.
38. Razin, S. The mycoplasmas.*Microbiol. Rev*., 1978; **42**: 414-470.
39. Razin, S., Knight, J.B. The effect of ribonucleic acid and deoxyribonucleic acid on the growth of mycoplasma.*J. Gen. Microbiol*., 1960; **22**: 504-519.
40. Quillent, C., Grau, O., Calvel, F., Montagnier, L., Blanchard, A. Inhibition of HIV type 1 reverse transcripatase assay by nucleases produced by contaminating mycoplasmas. *AIDS Res Hum. Retroviruses*., 1994; **10**: 1251-1257.
41. Shang, H., Miyakawa, Y., Sasaki, T., Nakashima, H., Ito, M. Suppression of HIV-1 reverse transcriptase activity by culture supernatants of mycoplasmas. *Microbiol.Immunol*., 1995; **63**: 4174-4177.
42. Paddenberg, R., Weber, A., Wulf, S., Mannherz, H.G. Mycoplasma nucleases able to induce internucleosomal DNA degradation in cultured cells possess many characteristics of eukaryotic apoptotic nucleases. *Cell Death Differ*., 1998; **5**: 517-528.
43. Paddenberg, R., Wulf, S., Weber, A., Heimann, P., Beck, L.A., Mannherz, H.G. Internucleosomal DNA fragmentation in cultured cells under conditions reported to induce apoptosis may be caused by mycoplasma endonucleases. *Eur. J. Cell Biol*., 1996; **69**: 105-119.
44. Sokolova, I.A., Vaughan, A.T., Khodarev, N.N. Mycoplasma infection can sensitize host cells to apoptosis through contribution of apoptotic-like endonuclease(s). *Immunol. Cell Biol*., 1998; **76**: 526-534.
45. Bendjennat, M., Blanchard, A., Loutfi, M., Montagnier, L., Bahraoui, E. Role of *Mycoplasma penetrans* endonuclease P40 as a potential pathogenic determinant. *Infect. Immun*., 1999; **67**: 4456-4462.
46. Zhang, S., Tsai, S., Wu, T.T., Li, B., Shin, J.W.K., Lo, S.C. *Mycoplasma fermentans* infection promotes immortalization of human peripheral blood mononuclear cells in culture. *Blood*., 2004; **104**: 4252-4259.
47. Liu, W., Shou, C. *Mycoplasma hyorhinis* and *Mycoplasma fermentans* induced cell apoptosis and vhanges in gene expression profiles of 32D cells. *Biol. Res*., 2011; **44**: 383-391.
48. Pollack, J.D., Hoffmann, P.J. Properties of the nucleases of mollicutes. *J. Bacteriol*., 1982; **152**: 538-541.
49. Schmidt, J.A., Browning, G.F., Markham, P.F. *Mycoplasma hyopneumoniae* mhp379 is a Ca2+ dependent, sugar-nonspecific exonuclease exposed on the cell surface. *J. Bacteriol*., 2007; **189**: 3414-3424.
50. II´inskaya, O.N., Sokurenko, Y.V., Ul´yanova, V.V., Vershinina, V.I., Zelenikhin, P.V., Kopakov, A.I., Medvedeva, E.S., Baranova, N.B., Davydova, M.N., Muzykantov, A.A., Chernova, O.A., Chernov, V.M. Ribonucleolytic activity of mycoplasmas. *Microbiologiia*., 2014; **83**: 320-327.
51. Cacciotto, C., Addis, M.F., Coradduzza, E., Carcangiu, L., Nuvoli, A.M., Tore, G., Dore, G.M., Pagnozzi, D., Uzzau, S., Chessa, B., Pittau, M., Alberti, A.*Mycoplasma agalactiae*MAG-5040 is a Mg2+-dependent,

sugar-nonspecific SNaserecognised by the host humoral response during natural infection.*PLoS One*., 2013; **8**:e57775.

52. Levitskiy, S.A., Sycheva, A.M., Kharlampieva,, D.D., Oberto, J., Kamashev, D.E., Serebryakova, M.V., Moshkovskii, S.A., Lazarev, V.N., Govorun, V.M.Purification and functional analysis of recombinant *Acholeplasmalaidlawii* histone-like HU protein.*Biochimie*., 2011; **93**:1102-1109.
53. Sharma, S., Tivendale, K.A., Markham, P.F., Browning,G.F.Disruption of the membrane nucleasegene (MBOVPG45-0215) of *Mycoplasmabovis*greatly reduces cellular nucleaseactivity.*J. Bacteriol*., 2015; **197**:1549-1558.
54. Xu, J., Teng, D., Jiang, F., Zhang, Y., El-Ashram, S.A., Wang, H., Sun, Z., He, J., Shen, J., Wu, W., Li, J.*Mycoplasmagallisepticum* MGA-0676 is a membrane-associated cytotoxic nuclease with a staphylococcal nuclease region essential for nuclear translocation and apoptosis induction in chicken cells.*Appl. Microbiol. Biotechnol*.,2015; 99:1859-1871.
55. Somarajan, S.R., Kannan, T.R., Baseman, J.B. *Mycoplasma pneumoniae* Mpn133 is a cytotoxic nuclease with a glutamic acid-, lysine- and serine-rich region essential for binding and internalization but not enzymatic activity.Cell *Microbiol*., 2010; **12**:1821-1831.
56. Li, L., Krishnan, M., Baseman, J.B., Kannan, T.R.Molecular cloning, expression, and characterization of a Ca2+-dependent, membrane-associated nuclease of *Mycoplasmagenitalium*.*J. Bacteriol*., 2010; **192**: 4876-4884.
57. Rivera, A., Cedillo, L., Hernandez, F., Giono, S. Desoxirrobonuclease activity in clinical *Mycoplasma fermentans* isolate. *Int. Res. J. Biol. Sci*., 2012; **1**: 82-84.
58. Kurinenko, B.M., Beliaeva, M.I., Cherepneva, I.E., Kuprianova-Ashina, F.G. Permeability of dextran-bound through the vascular barrier and tumor cell membrane. *Vopr.Onkol*., 1977; **23:** 86-90.
59. Kurinenko, B.M., Beliaeva, M.I., Cherepneva, I.E., Viesture, Z.A. Antitumor action of Serratiamarcescens nuclease covalently bound to soluble dextrans. *Vopr.Onkol*., 1977; **23:**94-98.
60. Black, K.L., Ciacci, J.D., Ammirati, M., Selch, M.T., Becker, D.P. Clinical trial of Serratiamarcescens extract and radiation therapy in patients with malignant astrocytoma. *J. Clin. Oncol*.1993; **11**: 1746-1750.
61. Desai, N.A., Shankar, V. Single-strand-specific nucleases. *FEMS Microbiol. Rev*., 2003; **26**: 457-491.
62. Gyles, C.L. Relevance in pathogenesis research. *Vet. Microbiol*., 2011; **153**: 2-12.
63. Henrich, B., Hopfe, M., Kitzerow, A., Hadding, U. The adherence-associated lipoprotein P100, encoded by an *opp* operon structure, functions as the oligopeptide-binding domain OppA of a putative oligopeptide transport system in *Mycoplasma hominis*.*J. Bacteriol*., 1999; **181**: 4873-4878.
64. Razin, S. Structure and function in mycoplasma. *Annu. Rev. Microbiol*. 1969; **23**: 317-356.
65. Wang, R.Y., Shih, J.W., Weiss, S.H., Grandinetti, T., Pierce, P.F., Lange, M., Alter, H.J., Wear, D.J., Davies, C.L., Mayur, R.K., Lo, S.C. *Mycoplasma penetrans* infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi´s sarcoma. *Clin. Infect. Dis*., 1993; **17**: 724-729.
66. Meens, J., Selke, M., Gerlach, G.F. Identification and immunological characterization of conserved *Mycoplasma hyopneumoniae* lipoproteins Mhp378 and Mhp651. *Vet. Microbiol*., 2006; **116**: 85-95.
67. Neale, G.A., Mitchell, A, Finch, L.R. Pathways of pyrimidine deoxribonucleotide biosynthesis in *Mycoplasma mycoides* subsp. *mycoides*. *J. Bacteriol*., 1983; **154**: 17-22.
68. Neale, G.A., Mitchell, A., Finch, L.R. Uptake and utilization of deoxynucleoside 5´-monophosphates by *Mycoplasma mycoides* subsp. *mycoides*. *J. Bacteriol*., 1984; **158**: 943-947.