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ROLE OF CHAPERONES IN BACTERIAL PATHOGENICITY - A NEW THERAPEUTIC STRATEGY

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ABSTRACT

There is an upsurge in the resistance pattern of bacterial pathogens demanding new therapeutic targets to keep in pace with the infectious organisms. This review discusses harnessing the virulence factors of microorganisms as drug targets that are produced to cope with demanding and rapidly changing environments during establishment in the host. It would be promising to develop small molecule inhibitors targeting specifically the stress proteins, so-called molecular chaperones that assist the protein folding machinery. There are several natural products that bind specifically to components of the chaperone machinery of microbial pathogens have been identified. Many successful pathogens have developed robust chaperone systems to conquer the stressful environments in the host environmental challenges, such as oxidative bursts that are often triggered in response to infection. Heat shock proteins are also linked for the emergence of drug resistance and hence targeting sites unique to the bacterial pathogens can be exploited for therapy, chaperons are already viewed as targets for many human ailments like protein aggregation diseases and cancer. This review discusses new insights in exploiting bacterial chaperones as drug targets and their role in pathogenicity.

Keywords: Hsp/HSP, heat shock protein; HspR, heat shock protein receptor; Csr1, cellular stress response protein; Cpx, extra cytoplasmic stress.

INTRODUCTION

Molecular chaperones from bacteria to humans are a highly conserved class of proteins and represent a significant proportion of the total protein content of all living cells. Many heat Shock Proteins function as molecular chaperones and perform important functions in protein folding, unfolding or translocation, assembly and disassembly of protein complexes, in reversing polypeptide unfolding, preventing protein aggregation, and repairing proteins that have been damaged or misfolded by stress (Craig, 1996). Under normal conditions, molecular chaperones are present at low concentrations in cells, but under stress conditions they accumulate to high levels (Kohler et al., 2002) and therefore enable cells to survive. Thus, chaperones are important in both normal and stressed cells. During infection, the molecular chaperones production increases in both pathogen and host cells [Steinhoff et al., 1994). When entering the host from the environment, a pathogen is confronted with several changes, some of which under stress due to temperature, pH and pO2 and changes the natural host resistance mechanisms, phagocytes

etc. The HSP expression in bacteria may be due to the factors like temperature changes, exposure to sub lethal concentrations of ethanol, heavy metals, oxidative stress and antibiotics (Laport et al., 2006; Yamaguchi et al., 2003). To establish successfully in a host, pathogenic bacteria depend on many critical factors at the molecular level that occurs at the interface between the host and pathogen. Gram negative bacteria's have specific adhesions known as pili or fimbriae and non pilus adhesion polymers to colonize on hosts with their extracellular proteinaceous appendage that are responsible for recognizing and binding to host specific receptors. They enable various benefits to the bacteria like host invasion, motility, biofilm formation and transport mechanisms (DNA and proteins). On the basis of biosynthetic pathways these non-flagellar appendages are categorized into five major classes among these assemble machineries, chaperone-usher pili (Sauer, 2004), are studied in extensively as they are involved in many Enterobacterial infections and other gram negative organisms. The pili composed of many subunits and are assembled on the outer membrane by two machinery systems, the periplasmic chaperone and an outer membrane pore forming usher. These unfolded proteins that reach the periplasmic space needs to be folded in proper conformation and targeted by the chaperons to the usher to be secreted on the outer membrane; if this mechanism fails, the pathogens will lose the colonizing capability on the host. The molecular details of how the subunit interacts with the chaperone and the importance of investigating therapeutics that specifically target the chaperone with low molecular weight compound which bind to bacterial chaperon could function as pilus assembly inhibitors in vivo are worth to study in detail.

Infection of a higher eukaryote by a bacterial parasite is a complex process involving a series of recognition events and phenotypic alterations, during which cells of the host and the bacteria undergo a process of mutual recognition and adaptation (Lathigra et al., 1991). HSPs were assumed to play a role in infection, since microbial HSPs can stimulate immune responses in infected patients (Misra et al., 1996). Indeed, some of the HSPs are themselves virulence factors, while others affect pathogenesis indirectly by increasing bacterial resistance to host defenses or regulating virulence genes (Gophna & Ron, 2003). Some bacteria are intracellular parasites and are able to invade eukaryotic cells to protect it against the host; the pathogen activates various evasion mechanisms, including increased production of molecular chaperones (Kohler, 2002). Bacterial chaperones play an important role in protein secretion, while indirectly contributing to bacterial virulence (Stebbins, 2005). There are several evidences which support the hypothesis that molecular chaperones of bacteria behave as direct virulence factors. Other effects of bacterial molecular chaperones on host cells include cell-cell signaling and promoting apoptosis (Henderson et al., 2006). In this paper, the involvement of bacterial chaperones in pathogenicity within the host and approaches to therapeutics is reviewed. Identification of new aspects of the bacterial chaperones involvement in the bacteria–host interaction will undoubtedly constitute a major step in understanding the molecular mechanisms developed by these organisms. Chaperone targeted drug designing will offer a promising therapeutic option in treating the multidrug resistant pathogenic microorganisms.

Modulation and regulation of bacterial chaperones for pathogenicity

Induction of heat shock protein genes in bacteria is either positively regulated through alternate sigma factors or negatively regulated by the transcription factor HspR (heat shock protein

receptor). Manipulation of either of these factors alters heat shock response and also influences bacterial virulence (Gophna and Ron, 2003). In the case of *Helicobacter pylorus*, survival in human gastric mucosa requires it to withstand constantly fluctuating environments and low pH conditions. *H. pylori* responds to environmental changes by modulating a regulator of heat shock protein genes called Csr1 (cellular stress response protein1; *hspR* is one of the genes modulated by Csr1). Consistent with the possibility that pathogens regulate stress responses to effective virulence, and *H. pylori* mutant for the *csr1* gene showed attenuated infection in a mouse model indicating that genes involved in stress tolerance are critical for *H. pylori* virulence (Barnard et al., 2004). However, in *Mycobacterium tuberculosis*, inactivation of *hspR* and the overexpression of Dna K cause an enhanced clearance of the bacterium in the mouse model of tuberculosis (Gupta et al., 2008). It appears that increased synthesis of DnaK primes the immune system early during infection possibly resulting in increased bacterial clearance. This example indicates that mere overexpression of heat shock proteins may not always confer virulence to bacteria as in the case with induction of heat shock proteins by fluoroquinolones in *E.coli* correlated well with DNA relaxation but not with cell death (Tohru Mizushima, 1997). Thus the timing and possibly the magnitude of expression needs to be regulated for their cytoprotective effects.

One of the best known examples of the role of heat shock proteins in bacterial pathogenesis is *Listeria monocytogenes* infection in host macrophages. After being phagocytosed into the host, *Listeria* can be digested upon fusion of the phagosome with an endosomal compartment. However, the bacteria rely on a member of Hsp100 family called ClpC to overcome this consequence. Expression of ClpC in the phagosome allows *Listeria* to be released from the phagosome into the host cytoplasm where it undergoes multiplication (Nair et al., 2000). Experiments with a *clpC* deletion strain of *Listeria* showed accumulation of the bacteria in phagosomes resulting in a significant reduction of bacterial load in a mouse model of infection. While the precise mechanism by which ClpC mediates bacterial exit from the phagosome is not known, it is possible that its interaction with phagosome membrane proteins may be involved. In *Brucella suis, Campylobacter jejuni* and *Salmonella enterica* serovar *Typhimurium*, deletion of DnaK results in compromised growth in macrophages or inability to colonize mice (Konkel et al., 1998). The role for another member of Hsp100 family, ClpB, was shown in *Francisella tularensis* infection of macrophages. ClpB was essential for *F. tularensis* to replicate in target organs and cause pathogenesis in mice (Melbom et al., 2008). These studies clearly indicate that intracellular pathogens rely upon their endogenous chaperone machinery to survive and establish an infection within their hosts. Besides these examples there are several reports that suggest a role for bacterial chaperones at the cell surface, as adhesions for invading the host cell or in signaling the immune system. Both the Hsp60 and Hsp70 classes of chaperones have been implicated in this role. In addition, there are also examples where bacteria have found ways to recruit or target host chaperones to enhance bacterial growth and overcome host defense (Henderson et al., 2006). It is apparent that bacteria have mainly exploited their heat shock protein functions to cope with host defense mechanisms triggered in response to infections; additional roles in invasion and multiplication have also been suggested in some of the cases.

Antibiotic stress for the induction of chaperones

Resistance to antibiotics typically occurs as a result of drug inactivation or modification, target

alteration or reduced accumulation associated with decreased permeability and/ or increased efflux. The majority of these mechanisms of resistance depend on the rapid over expression of different kinds of protein (Peleg et al., 2008). Challenge of *S. aureus* with cell wall-active antibiotic initiates an extensive program of gene and protein expression. A large number of genes, including ones encoding proteins involved in cell wall metabolism and stress responses were up regulated by oxacillin, D-cycloserine or bacitracin. This response may represent the transcriptional signature of a cell wall-stress stimulon induced in response to cell wall-active agents. An insertional inactivation in the middle of DnaK, a member of the cell wall stress stimulon, using a kanamycin resistance gene, in *S. aureus* strains RN450, SH1000, and COL resulted in mutants that grew poorly at temperature above 45°C (Utaida, 2003). DnaK increased more than fourfold after 60 min of exposure to a subinhibitory concentration of antibiotic, and GroEL levels doubled. Furthermore, *Acinetobacter baumannii* cells pretreated for 30 min at 45°C had an increased ability to survive antibiotic exposure compared with cells pretreated at physiological temperatures. These results suggest that the chaperones DnaK and GroEL could play an important role in the stress response caused by streptomycin in *A.baumannii*. (Karen Cardoso, 2010).

In *S. aureus*, the Hsp100/Clp ATPases have been extensively studied and they have been shown to play important roles in stress tolerance, intracellular replication in eukaryotic epithelial cells, biofilm formation, expression of extracellular toxins, and pathogenicity in a murine model of infection (Chatterjee et al., 2005). The lack of a functional DnaK reduces oxacillin and methicillin tolerance in *S. aureus*. The mutation in DnaK had increased the susceptibility of methicillin-resistant strain COL to the cell-wall-active antibiotics oxacillin and methicillin. In the case of the methicillin-susceptible strain SH1000, deletion of DnaK did not reduce the oxacillin MIC, but it had led to a significantly reduced survival after oxacillin treatment. The decreased oxacillin MIC of the DnaK mutant of strain COL, and the decreased persistence of the DnaK mutant of strain SH1000, suggested that protein damage does occur as a result of challenge with cell-wall active antibiotics, and that DnaK plays a role in dealing with these damaged proteins. In *Staphylococcus aureus*, a mutation in the DnaK gene increased the susceptibility of the methicillin-resistant strain COL to the antibiotics oxacillin and methicillin (Singh *et al*., 2007). In *S. aureus*, HSPs are thought to be involved in responses to antibiotics because they are induced when the cell wall is subjected to antibiotic stress (Utaida et al., 2003). HSPs are up regulated at the transcriptional and translational levels by different kinds of antibiotic and that this up regulation could improve the ability of the cell to cope with stress caused by antimicrobials and perhaps acts simultaneously with the antibiotic resistance machinery to maintain cell survival. Some studies have suggested that the role for HSPs in the bacterial stress response due to antibiotics. Yamaguchi et al., 2003 demonstrated that the chaperones DnaK and GroEL have an effect on the antimicrobial activity of the fluoroquinolone, levofloxacin in *E. coli* and briefed that chaperones might contribute to quinolone resistance because they sequester the aggregates that accumulate in cells exposed to fluoroquinolones. Furthermore, mutations in the DnaK, GroEL and Lon genes increased bacterial susceptibility to levofloxacin. Recent studies have demonstrated that sub inhibitory concentrations of the antimicrobials gentamicin and tobramycin induced a set of genes that affect the interaction of *Pseudomonas aeruginosa* with

host cells, including the gene encoding Lon protease, which is known to play a role in protein quality control (Marr et al., 2007). Sparfloxacin induces DnaK and GroEL proteins, the major heat shock proteins of *E. coli*, in a dose-dependent manner.

A large number of genes, including ones encoding proteins involved in cell wall metabolism and stress responses were up regulated by oxacillin, D-cycloserine or bacitracin. Penicillin dose-dependently increased *clpL* levels but decreased *pbp2x* levels, because ClpL is induced by heat shock and other stresses (Kwon et al., 2003), pneumococcal survival seems to depend on competition between the amounts of ClpL and PBP2x. Low-level stress may induce ClpL and increase cell resistance to penicillin compared to normal cells and hence low-level stress could improve pneumococcal survival. This implies that treatment with cell wall-active antibiotics results in damage to proteins. These changes in gene expression can be viewed as an attempt by the organism to defend it against the antibacterial activities of the agents. The molecular chaperone DnaK has been shown to be induced by a variety of stresses including cell wall-active antibiotic stress, suggesting its potential role in response of bacteria to stresses.

Pilus biogenesis and host pathogenesis

Colonization is not a single event but rather a dynamic process that involves panoply of changes in both the bacterium and host alike as a result of attachment. (Mulvey et al.,1998) have recently found during interactions between type 1-piliated *E. coli* and host superficial epithelial bladder cells glycoproteins, pili shortened to an average length of 0.12 to 0.01 mm compared to broth culture where they were 1 to 2 mm long. The mechanism by which this apparent shortening occurs remains unknown, but retraction of the pilus upon attachment has been suggested as one possible means (Mulvey et al., 1998). Scanning and high-resolution electron microscopy has shown that type 1 pili can mediate direct and intimate bacterial contact with the uroplakin coated bladder epithelium. Environmental conditions in the urinary tract presumably favor the expression of pili at critical points during the pathogenic cascade (Lim et al., 1998). Interestingly, pilus assembly leads to the activation of Cpx (extra cytoplasmic stress), which in turn would serve to reinforce the commitment to make pili by activating periplasmic assembly factors and maintaining the phase variation in ON state. This would ensure that daughter cells remain piliated in order to facilitate the colonization of the epithelium and persistence in the urinary tract. In addition, host–pathogen interactions in the urinary tract activate a cascade of innate defenses, such as an increase in the production of nitric oxide that may induce the periplasmic stresses (Mumtaz et al., 2000) and lead to an activation of Cpx. The activation of Cpx via pilus biogenesis and host–pathogen interactions serves to reinforce the commitment to produce pili, facilitating colonization of the urinary tract. In addition, Cpx may control the expression of a number of other virulence factors, putative CpxR binding sites upstream of genes encoding hemolysin, cytotoxic necrotizing factor and type 1 pili (Hung *et al*., 1996). Thus, environmental factors that activate pilus expression may indirectly activate additional virulence factors due to the induction of Cpx by OFF-pathway subunits. In this way, Cpx may tie up the expression of virulence factors to pilus biogenesis, which may be part of a mechanism in which microbial colonization is linked to the expression of new genes important in the pathogenic cascade and establishment in the host. The type 1 pilus adhesin FimH binds to mannose-containing receptors expressed by many host cell types and is a significant virulence determinant in the development of

bladder infections (Thankavel, 1997). It has been shown that uropathogenic *E. coli* and K12 strains expressing type 1 pili can invade cultured human bladder epithelial cells, whereas non-piliated strains or piliated strains lacking the FimH adhesin cannot. Thus, FimH may function like the invasin of *Yersinia pseudotuberculosis* and the internalin of *Listeria monocytogenes* (Parida, 1998). Once internalized, type 1-piliated *E. coli* cystitis isolates, but not their K12 counterparts, are able to proliferate and survive within the host cell. Thus the elucidation of the molecular mechanisms of pilus mediated bacterial attachment to host cells and the consequent pathogenesis offers the opportunity to develop new methods of prevention and treatment for many bacterial diseases.

Drug targeting based on the mechanism of the chaperone –usher system

Strategies for inhibition of pilus biogenesis are based on three different points of the processes of pilus biogenesis and adhesion: inhibition of chaperone-subunit complex formation; inhibition of chaperone-subunit interaction with the usher; and inhibition of adhesion to the host receptor. P and type 1 pili are responsible for the early onset and persistence of UPEC-caused urinary tract infections (UTIs) by mediating attachment to the kidney epithelium (P pili) or attachment and invasion of the bladder epithelium cells (type 1 pili), respectively. They are assembled by the conserved chaperone/usher (CU) pathway, responsible for the biogenesis of more than 100 surface organelles in many other important human pathogens (*Yersinia, Salmonella, Shigella, and Haemophilus*). The activity of a family of bicyclic 2-pyridones, termed pilicides, was evaluated in two different pilus biogenesis systems in uropathogenic *Escherichia coli*. A group of antibacterial agents called “pilicides” (pyridinones derivatives) was designed based on the crystallographic data of both PapD and FimC chaperones and their complexes (Svensson *et al*., 2001). Pilicides blocks the process of pilus biogenesis by binding to three possible binding sites in FimC or PapD. Inhibition of the latter interaction is particularly promising as it is the only one to have been convincingly established (Pinkner et al., 2006). Pilicides targeting this site, by inhibiting pilus biogenesis, have been shown to interfere with bacterial attachment and biofilm formation. Finally, anti-adhesive compounds targeted against the adhesive sub-units like anti-adhesin antibodies have been shown to block bacterial adhesion (Langermann, 2000), thus paves the way to antimicrobial vaccine development strategies.

Pilicides block pilus biogenesis by preventing chaperone-subunit complexes from interacting with the outer membrane usher (Pinkner et al., 2006). Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria. The crystal structure of a pilicide-chaperone complex indicates that the pilicide binds to a conserved hydrophobic patch on strands F_1, C_1 and D_1 on the back of the N-terminal domain of the pilus chaperone (Pinkner, et al.2006). So far, efforts at developing pilicides have focused on inhibiting the periplasmic chaperone proteins of the chaperone usher pathway in uropathogenic *E. coli* (UPEC). Bicyclic 2-pyridones and N-substituted amino acid derivatives have been shown to competitively inhibit binding of chaperones to pilin subunits by surface plasmon resonance (Svensson et al., 2001). *In vitro*, bicyclic 2-pyridones have also been shown to inhibit hemagglutination and biofilm formation in laboratory and clinical *E. coli* strains, and *ex vivo* they have been shown to inhibit adhesion of the bacteria to bladder carcinoma cells by ~90%. It has been suggested that pilicides may have broad-spectrum of activity due to the conservation of both chaperone structure and the chaperone-usher pathway (Lee, et al., 2003).

This is suggested as an alternative and attractive approach to meet the vital needs in developing new anti infective agents. The pilicides were designed to mimic the C-terminal part of the fimbrial subunits and to bind to FimC or PapD thus blocking the chaperone from binding to the pilus subunits in the periplasm. Another interaction site of these pilicides has been shown to be at the surface of the chaperone involved in the interaction with the usher, thus blocking subunit delivery. In addition to rationally designed small synthetic molecules, natural compounds are also screened for their activity against adhesion. Proanthocyanidines, a component of cranberry juice represents one of the most well known natural products used to block adhesion of uropathogenic E. coli in the bladder. Coating of abiotic surfaces with isolated group II capsule polysaccharides of E. coli has been shown to drastically decrease adhesion and biofilm formation and may be used for new strategies to reduce biofilm formation on medical devices (Valle, 2006).

Heat shock protein as antibody triggers and potential vaccines:

Analysis of immune responses to bacteria in the 1970s enabled scientists to identify what was termed as "common antigen" in many bacterial species. Patients infected with *Mycobacterium tuberculosis* or *Mycobacterium leprae* exhibits significant antibody responses to a 65-kDa antigen which was identified as the molecular chaperone Cpn60. It has now been established that a number of molecular chaperones from bacteria and protozoan parasites (Cpn60, Hsp70, and Hsp90) are (i) potent immunogens, (ii) active immunomodulators, and (iii) inducers of cross-reactive immunity and autoimmunity (Van Eden et al., 2005). The mammalian immune system recognizes the molecular chaperones of infecting parasites as strong immunological signals, which is surprising in view of the significant homology between host and parasite proteins. The Cpn60 (Hsp65) protein of *M. tuberculosis* has also proven to be an extremely powerful immunomodulator that is able to protect against a number of experimental autoimmune diseases in rodents, including diabetes. Members of the hsp70 family in other parasites and bacteria have proved to be immunogenic in both humans and experimental animals. For example, the hsp70 of *Schistosoma mansoni* shows about 85% homology with human hsp70, yet the majority of infected animals and humans develop antibodies to the *S. mansoni* protein (Hedstrom et al., 1987). The antibodies are directed towards the nonconserved sequences of the protein and can distinguished between infection with *S. mansoni* and that with *S. japonicum* (Scallon, 1987). Antibodies produced against malarial hsp70 proteins are directed mainly at nonconserved epitopes (Peterson, 1988), although a minor component also reacts with human hsp70 (24). Infection with *Brugia malayi* stimulates anti-hsp70 antibodies that react predominantly with epitopes specific to the Brugia protein, but there is some cross reactivity with hsp70 molecules from *S. mansoni* and *Plasmodium falciparum* (Selkrik *et al*., 1989). A 75-kDa protective antigen of *Chlamydia trachomatis* has also been identified as a member of the hsp70 family, and human antibodies react primarily with non conserved epitopes of the protein. Therefore, members of the hsp70 family from a wide range of parasites and bacteria are strong B-cell antigens, stimulating antibodies directed mainly towards the nonconserved regions of the protein. There is a variable degree of cross-reactivity with hsp70 proteins of other species and a minimal response to human hsp70, consistent with the need to preserve self tolerance. In the case of *M. leprae* hsp70, the dominant antibody response is directed to the C-terminal region.

Hsp60 has been found to be a common antigen of many bacterial pathogens including species of

Pseudomonas, Mycobacterium, Borrelia, Salmonella, Legionella, Coxiella and *Rickettsia* (Shinnick, 1991). In *Borellia burgdorferi,* there are two HSP60 of molecular masses 60 and 66 kDa which have been implicated in developing autoimmune pathologies such as arthritis (Carreiro *et al* 1990). HSP60 class of proteins serves as an immunodominant targets of α, ß and *γ, δ* classes of T-lymphocytes and have been used to provoke immunological protection against *Mycobacterium* and *Legionella* (Silva and Lowrie, 1994). It has been postulated that since HSP60 is highly conserved, the host may frequently encounter this antigen through infection with various other microorganisms there by constantly boosting the immune response to HSP (Kaufmann *et al* 1991). Chimeric pili/fimbriae with inserted antigenic sequences can be used as effective and safe recombinant vaccines, application of atypical fimbriae Saf for design of Salmonella vaccine components of the Salmonella atypical fimbriae (Saf) are investigated for inclusion in a Salmonella vaccine. Vaccination with purified Dr fimbriae reduces mortality associated with chronic urinary tract infection due to *E. coli* bearing Dr adhesin (Pawel Goluszko, 2005). Purified *E. coli* Dr fimbrial antigen to vaccinate C3H/HeJ mice against an experimental urinary tract infection due to a homologous strain bearing Dr adhesion had demonstrated reduced mortality in the vaccinated animals. Antipneumococcal DnaK antiserum did not cross react with DnaK homologues in *E.coli, staphylococcus aureus* and human cells and hence may be a good candidate as a vaccine (Hamel et al., 1997). Still, the role Dna K in pathogenesis remains unknown. The difference between gram positive, and gram negative bacteria, suggests that the regulation mechanism of heat shock response of gram positive bacteria (especially DnaK function of gram positive bacteria) is quite different than *E.coli* and other gram negative.

CONCLUSION

The new paradigm for antimicrobial therapy should redefine the goal of balance in favor of the host enabling to control infection, rather than complete *in vivo* killing of a pathogen by the drug itself. Though some virulence inhibitors such as pilicides and T3SS inhibitors have the potential to target a wider spectrum of bacteria, new approaches have to be designed to understand how bacteria cause disease. As antibiotic resistance continues to evolve and the need for new antimicrobials continues to grow therapeutics that target *in vivo* essential gene functions, all the more compelling. Pilicides, by blocking chaperone and usher functions, have the potential to inhibit pili formation in a broad spectrum of pathogenic bacteria to prevent critical host–pathogen interactions necessary for many diseases. There are hundreds of diverse cell-surface virulence organelles that are assembled by the chaperone–usher pathway in important bacterial pathogens. Because all chaperones have a conserved structure and mechanism of action, it is reasonable to propose that pilicides likely have broad-spectrum activity. These pilicides represent an example of selective, low-molecular-weight, nonpeptidic virulence-determinant inhibitors (Gauthier *et al*., 2005; Hung et al., 2005). Immunization with a FimH-based vaccine reduces bladder colonization by uropathogenic *E. coli* by 99% in a mouse cystitis model and suggested adhesin-based vaccines may be effective in preventing both urinary tract and other bacterial infections. Understanding the molecular events involved in the biogenesis of these organelles will be crucial for the development of novel therapeutic strategies. Elucidating common themes in these pathways will be a prerequisite for any efforts targeted towards developing a therapeutic

strategy with broad-spectrum activity. Targeting bacterial virulence or disrupting the interaction between the host and the pathogen are attractive options and should be explored. The identification of those processes that occur following attachment will undoubtedly open up further avenues of therapeutic possibilities, as we come closer to understanding how host-pathogen interactions lead to the expression of bacterial genes that are important in pathogenesis.

Besides having high sequence homology Hsps can be used as protective vaccines. The design of new drugs can be based on either mimicking the conformation of known ligands or on the structure of the peptide-binding domain of the receptor. The IgG repertoire during intravesical bacille Calmette- Guerin (BCG) immunotherapy in superficial bladder patients includes antibodies to GroEL and Hsp70 but not to DnaK (Zlotta et al., 1997) exploiting the functional differences between bacterial DnaK and the analogous human Hsp70. This finding emphasizes the significant structural and functional differences between mammalian Hsp70 and the corresponding bacterial DnaK and supports our findings and hypothesis that antibacterial peptides can inactivate DnaK (bacterial Hsp70) without binding to Hsp70 (human) and affecting its normal functions. Drosocin, pyrrhocoricin, and apidaecin, representing the short (18-20 amino acid residues) proline-rich antibacterial peptide family, originally isolated from insects, were shown to act on a target bacterial protein in a stereospecific manner. Radicicol represents the first non –benzoquninone ansamycin antifungal inhibitor of Hsp90. It is thus worth investing both effort and cost effective in antivirulence inhibitor research. In this effort, structural biology allied with improved computational tools provides a powerful platform for rational chemical design and thus, should help fulfill our antibiotics development goals in the very near future. In conclusion microorganisms have smartly harnessed utilization of host's unfavorable environment by several molecular mechanisms, increased induction of genes coding for stress proteins and deletion of these genes resulted in attenuation of their growth within the host. Inhibitions of these factors are attainable by pharmacological new lead compounds that function directly by binding and inhibiting HSPs are under clinical trials are cited in literatures.

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