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Holins: Proteins of Diverse Function with Potential for Biomedical and Biotechnological Advances

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Mini Review

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INTRODUCTION

Holins are conserved viral and cellular proteins that elicit a variety of effects and serve various functions [1]. Their best studied function involves bacterial cell lysis, a process used by double stranded DNA phages to form open or tightly sealed channels in the host bacterial cell membrane, releasing an autolysin and ultimately, following lysis of the cell, releasing the phage progeny [2-4]. At a precise, highly regulated time during phage development, these hydrophobic membrane proteins spontaneously oligomerize in the bacterial cytoplasmic membrane, forming large, nonspecific, transmembrane "holes" that reduce the membrane potential and release endolysins that hydrolyze specific bonds in the peptidoglycan cell wall [5]. This allows the release of completely synthesized virions following viral multiplication at the expense of host bacterial cell catabolism after the cell has been parasitized by the phage.

Not all phage holins function in the same mechanistic way. For example, pinholins, such as the one identified in lambdoid phage 21, depolarizes the membrane by forming small holes, activating a signal that regulates release of the endolysin [6]. Holins form transmembrane pores as solo agents, rarely fusing to or functioning with other proteins, and the holes formed are homooligomeric transmembrane structures with variable numbers of subunits, depending on the size of the hole required for export of the endolysin (**Figure 1**). Moreover, these proteins exhibit variable numbers of transmembrane alpha-helical segments (TMSs), (between 1 and 4 TMSs), depending on the holin (**Figure 2**). Some holins have originated as small 2 TMS proteins and duplicated internally during evolutionary time to yield 4 TMS proteins that are about twice as large [7]. It is thought that the pore adds and eliminates subunits while shaping itself around the endolysin as it passes through the membrane [8,9].

Despite their large sequence and topological diversity, all holins have an N-terminal transmembrane region and a highly charged C-terminal region [10]. Fifty-nine sequence divergent families of holins and putative holins are tabulated, and twenty-two of these families cluster into seven apparently phylogenetically unrelated groups or superfamilies as categorized in the Transporter Classification Database (TCDB; www.tcdb.org) [11,12]. Thus, Superfamilies I - VII include 1, 5, 7, 4, 2, 2 and 1 families, respectively, and the two superfamilies with only one family include multiple sequence divergent sub-families (3 and 6 subfamilies for superfamilies I (family with TC#1.E.11) and VII (family with TC#1.E.36), respectively [7].

Holins have been identified in numerous free living and pathogenic bacterial species. These include members of numerous phyla including Proteobacteria, Firmicutes, Spirochetes, Bacteroidetes, Actinobacteria, Chloroflexi, Cyanobacteria, Deinococcus/Thermus, Fusobacteria, Planctomycetes, Tenericutes, Thermatogae and Verrucomicrobia [8], with the best characterized deriving from Proteobacteria and Firmicutes. Pathogenic bacterial species such as those found in the genuses (and their phage) of Lepto-

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spira [13], Bacillus [14], Clostridium [15], Staphylococcus [16] and Streptococcus [17] are often effective as anti-bacterial agents. These holins vary from one to four TMSs and sizes that are roughly proportional to the numbers of TMSs (see **Figure 2**) [8]. It has been noted that holins are very rarely fused to other protein domains, suggesting that they form transmembrane pores as homooligomers without the participation of other proteins or protein domains [8]. This conclusion has been established for several of the best-characterized holins.

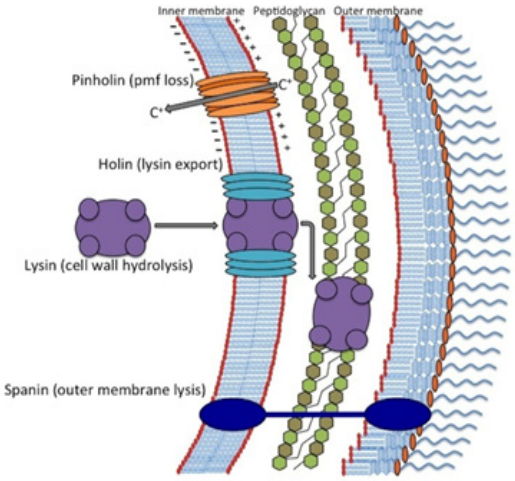

Figure 1: Schematic depiction of the proteins involved in Gram-negative bacterial cell envelope disruption by holin-type lysis systems. Pinholins form small heptameric pores that collapse the membrane potential (the PMF) across the inner membrane, while the more conventional holins form large multi-subunit pores of variable sizes that allow release of fully folded endolysins from the cytoplasm, which in the periplasm hydrolyze specific bonds in the peptidoglycan cell wall, depending on the lysin type. One- or two-component spanins disrupt the outer membrane by an unknown mechanism, possibly involving fusion of the outer membrane with the inner membrane. C+, a cation.

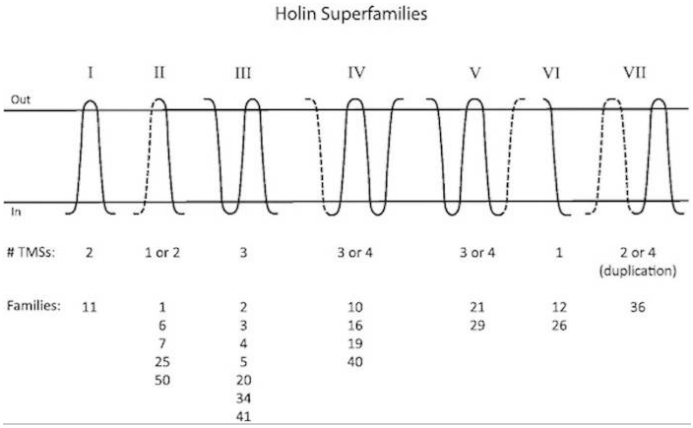

Figure 2: Schematic view of the topologies of members of the seven holin superfamilies (I to VII). Solid lines represent TMSs that are present in all known members of the superfamily. Dashed lines represent TMSs present in some but not all members. # TMSs, the number of transmembrane α-helical segments (TMSs) predicted for the proteins of a superfamily. Family numbers refer to the families in subclass 1.E of the Transporter Classification Database.

Holins are not only found encoded in phage genomes; they are also encoded by bacterial, archaeal and eukaryotic genes [1]. Saier and Reddy reviewed the various functions apparently served by these proteins [1]. These functions include [1] intercellular gene transfer, [2] biofilm formation, [3] spore morphogenesis, [4] DNA release, [5] toxin export, [6] programmed cell death, [7] oxidative stress adaptation, and [8] possibly, control of acetate metabolism. Some of these functions, such as programmed cell death, have probably been transmitted to eukaryotes. See Saier and Reddy, 2015 for references to the original research articles describing the roles of holins and potentially related proteins in these cellular processes.

Phage and bacterial cell holins have been reported to exert antibacterial activity against heterogenous pathogens [16,18-20] as well as antiviral activity [21]. Holins alone, without endolysins, compromise the cell membrane but are unable to destabilize the cell wall. Thus, peptidoglycan destruction, mediated by the holin-exported endolysin, is necessary for complete cell lysis.

Additional proteins, called spanins (**Figure 1**), are necessary for cell lysis in osmotically stabilizing environments [22,23]. *E. coli* cells infected with λ phage without an endolysin gene do not lyse, but cells lose their membrane potential and their ability to respire; they become “leaky” to normally impermeant molecules [24]. A holin-like protein, Tmp 1, has been reported to have bacteriostatic and membrane damaging properties [25]. The ability of Tmp 1 to damage the cell membrane is associated with its N-terminal

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hydrophobic transmembrane domain, typical of holins [25]. Such studies provide strong evidence that holin-mediated destruction of cell membranes, alone or together with autolysins and spanins, can be used to cause antibacterial and bacteriostatic effects, serving as an alternative to the use of antibiotics [26].

The combined use of holins and endolysins may be an effective strategy to cure infections and prevent bacterial resistance to antibiotics. Common bacteriophages [27], virophages [28], mycophages [29], zymophages [30] and cyanophages [31] provide successful and promising alternative therapies against infections caused by bacteria. They can also function in food safety against fungal infections, prevent resistance against zoonotic infections and be used to protect our aquatic environment [32,33].

To summarize, holins, with or without their companion autolysins and spanins, serve useful functions to bacterial cells and are required for the successful propagation of numerous viruses in bacterial hosts. They additionally have tremendous potential in combating bacterial drug resistance and have been invaluable in the development of biotechnological advances [1]. We are likely to discover many additional functions and uses for these proteins as further research on these amazing proteins advances.

REFERENCES

1. Saier MH J, Reddy BL. Holins in bacteria, eukaryotes, and archaea: multifunctional xenologues with potential biotechnological and biomedical applications. J Bacteriol. 2015;197:7-17.
2. Blasi U and Young R. Two beginnings for a single purpose: the dual-start holins in the regulation of phage lysis. Mol Microbiol. 1996; 21: 675-682.
3. Young R and Blasi U. Holins: form and function in bacteriophage lysis. FEMS Microbiol. 1995; Rev. 17:191-205.
4. Young R. Bacteriophage lysis: mechanism and regulation. Microbiol Rev. 1992;56:430-481.
5. Wang IN, et al. Holins: the protein clocks of bacteriophage infections. Annu Rev Microbiol. 1994;54:799-825.
6. Park T, et al. The pinholin of lambdoid phage 21: control of lysis by membrane depolarization. J Bacteriol. 2007;189:9135-9139.
7. Reddy BL and Saier MH Jr. Topological and phylogenetic analyses of bacterial holin families and superfamilies. Biochim Biophys Acta. 2013;1828:2654-2671.
8. Kuppusamykrishnan H, et al. Analysis of 58 Families of Holins Using a Novel Program, PhyST. J Mol Microbiol Biotechnol. 2016; 26: 381-388.
9. Wang IN, et al. Sizing the holin lesion with an endolysin-beta-galactosidase fusion. J Bacteriol. 2003;185:779-787.
10. Sandberg K, et al. Cholinergic lesion of the striatum impairs acquisition and retention of a passive avoidance response. Behav Neurosci. 1954;98:162-165.
11. Saier MH, et al. The Transporter Classification Database: recent advances. Nucleic Acids Res. 2009; 37: D274-278.
12. Saier MH, et al. TCDB: the Transporter Classification Database for membrane transport protein analyses and information. Nucleic Acids Res. 2006;34:D181-186.
13. Buyuktimkin B and Saier MH. Comparative analyses of transport proteins encoded within the genomes of Leptospira species. Microb Pathog. 2016;98:118-131.
14. Kong M and Ryu S. Bacteriophage PBC1 and its endolysin as an antimicrobial agent against Bacillus cereus. Appl Environ Microbiol. 2015; 81:2274-2283.
15. Govind R, et al. 2015. Observations on the Role of TcdE Isoforms in Clostridium dificile Toxin Secretion. J Bacteriol. 197:2600-2609.
16. Song J, Xia F, Jiang H, Li X, Hu L, Gong P, et al. 2016. Identification and characterization of HolGH15: the holin of Staphylococcus aureus bacteriophage GH15. J Gen Virol. 97:1272-1281.
17. Garcia P, Martin AC, Lopez R. 1997. Bacteriophages of Streptococcus pneumoniae: a molecular approach. Microb Drug Resist. 3:165-176.
18. Roach DR, Donovan DM. 2015. Antimicrobial bacteriophage-derived proteins and therapeutic applications. Bacteriophage. 5:e1062590.
19. Jonczyk-Matysiak E, et al. Possible use of bacteriophages active against Bacillus anthracis and other B. cereus group members in the face of a bioterrorism threat. Biomed Res Int. 2014:735413.
20. Hraiech S, et al. Bacteriophage-based therapy in cystic ibrosis-associated Pseudomonas aeruginosa infections: rationale and current status. Drug Des Devel Ther. 2015;9:3653-3663.

e-ISSN:2320-3528
p-ISSN:2347-2286

21. Bakhshinejad B and Sadeghizadeh M. Bacteriophages and their applications in the diagnosis and treatment of hepatitis B virus infection. World J Gastroenterol. 2014;20:11671-11683.

22. Young R. Phage lysis: three steps, three choices, one outcome. J Microbiol. 2014;52:243-258.

23. Young R. Phage lysis: do we have the hole story yet? Curr Opin Microbiol. 2013;16:790-797.

24. Young R. Bacteriophage holins: deadly diversity. J Mol Microbiol Biotechnol. 2002;4:21-36.

25. Rajesh T, et al. Functional characterization of a new holin-like antibacterial protein coding gene tmp1 from goat skin surface metagenome. Appl Microbiol Biotechnol. 2011;89:1061-1073.

26. Sharma M. Lytic bacteriophages: Potential interventions against enteric bacterial pathogens on produce. Bacteriophage. 2013;3:e25518.

27. Hatfull GF and Hendrix RW. Bacteriophages and their genomes. Curr Opin Virol. 2011;1:298-303.

28. Koonin EV and Krupovic M. Polintons, virophages and transpovirons: a tangled web linking viruses, transposons and immunity. Curr Opin Virol. 2017;25:7-15.

29. Emery DL and Whittington RJ. An evaluation of mycophage therapy, chemotherapy and vaccination for control of Mycobacterium avium subsp. paratuberculosis infection. Vet Microbiol. 2004;104:143-155.

30. Lindegren CC. Progress report on the zymophage. Trans N Y Acad Sci. 1962;24:540-566.

31. Gao EB, et al. Metabolic Genes within Cyanophage Genomes: Implications for Diversity and Evolution. Genes (Basel). 2016;1:7.

32. Schmelcher M, et al. Bacteriophage endolysins as novel antimicrobials. Future Microbiol. 2012;7:1147-1171.

33. Cisek AA, et al. Phage Therapy in Bacterial Infections Treatment: One Hundred Years after the Discovery of Bacteriophages. Curr Micro. 2017;74:277-283.