to kill persister and stationary cells; their effectiveness on the problematic biofilm arrangement of pathogenic bacteria; the absence of cross-resistance with antibiotic resistance mechanisms and thus their effectiveness on multidrug-resistant strains; and the immutable nature of peptidoglycan that turns highly unlikely the development of resistance, among many others. Even so, the existence of an OM in Gram-negative cells has impaired their use on these bacteria and their efficient use will almost always require the use of OMPs. We showed here that some strategies have been developed, turning the use of endolysins effective and efficient against problematic and resistant pathogenic Gram-negative bacteria. Considering the strategies presented, we believe that those based on protein engineering will rise and be established as the most promising strategy for therapeutic application since it will result in a completely protein-based product, most likely biodegradable, and thus with low probability to accumulate in the environment and with a lower probability of inducing resistance in bacteria, contrasting with many persisting antibiotics.

15.4 Holins

Both Gram-positive and Gram-negative bacteria present an inner membrane in their cell structure that separates and impairs the contact of the intracellular content with the peptidoglycan of the cell wall. Consequently, the endolysin that accumulates during phage replication to produce cell lysis and the release of the progeny phages usually cannot have access to its substrate per se. It is here where holins exert their main function. Holins are small hydrophobic membrane proteins that accumulate in the membrane. After reaching a genetically defined threshold concentration, they are triggered to form holes. These "time-programmed" holes are usually large enough to allow the passage of the fully folded endolysins and its contact with the peptidoglycan. The consequences are the digestion of the peptidoglycan and the bursting of the cell with the release of the phage progeny (Figure 15.1i) (Smith et al. 1998; Dewey et al. 2010; Fernandes and São-José 2018).

15.4.1 Holin Structure

Holins constitute one of the most diverse functional groups, and 52 families of established or putative holins have been identified. Experimental topological analyses suggest that these small proteins span the cytoplasmic membrane from one to four times as transmembrane α-helical segments. Within the same family, the protein size and topology are usually conserved and they have been identified in phages infecting both Gram-negative and Gram-positive bacteria (Reddy and Saier 2013; Saier and Reddy 2015). The high diversity in holin