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Colicin import into *E. coli* cells: A model system for insights into the import mechanisms of bacteriocins

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ABSTRACT

Bacteriocins are a diverse group of ribosomally synthesized protein antibiotics produced by most bacteria. They range from small lanthipeptides produced by lactic acid bacteria to much larger multi domain proteins of Gram negative bacteria such as the colicins from *Escherichia coli*. For activity bacteriocins must be released from the producing cell and then bind to the surface of a sensitive cell to instigate the import process leading to cell death. For over 50 years, colicins have provided a working platform for elucidating the structure/function studies of bacteriocin import and modes of action. An understanding of the processes that contribute to the delivery of a colicin molecule across two lipid membranes of the cell envelope has advanced our knowledge of protein–protein interactions (PPI), protein–lipid interactions and the role of order–disorder transitions of protein domains pertinent to protein transport. In this review, we provide an overview of the arrangement of genes that controls the synthesis and release of the mature protein. We examine the uptake processes of colicins from initial binding and sequestration of binding partners to crossing of the outer membrane, and then discuss the translocation of colicins through the cell periplasm and across the inner membrane to their cytotoxic site of action.

1. Introduction

Within the ecological niche of highly diverse communities, there is often fierce competition between various micro-organisms such as bacteria, fungi and Archaea for resources and space. Many species have evolved to produce a huge diversity of antimicrobial molecules that can either inhibit (bacteriostatic) or kill (bacteriocidal) competitor species including broad-spectrum antibiotics, proteinaceous exotoxins, lysozymes, and bacteriocins [1]. Since the discovery of the first bacteriocin by André Gratia nearly 90 years ago from *Escherichia coli* [2], bacteriocins have been found in nearly all bacterial lineages and some Archaea and are now regarded as the most diverse and naturally abundant classes of antimicrobial molecules [1,3,4]. Gratia's bacteriocin was termed a 'colicin' after the species name of the producing organism. A similar system of nomenclature was adopted for most bacteriocins discovered thereafter such as pyocins from *Pseudomonas aeruginosa* (formerly *Pseudomonas pyocyanea*), klebicin from *Klebsiella pneumoniae*, diffocins from *Clostridium difficile*, epidermin and gallidermin from *Staphylococcus epidermidis* and *S. gallinarum* respectively, and the newly discovered thuricin 17 from *Bacillus thuringiensis*. Despite huge differences in chemical structures and post-translational processing of bacteriocins between the small (un)modified lanthipeptides produced by lactic acid bacteria and the much larger multi domain polypeptides produced by Gram negative bacteria such as the colicins, all bacteriocins are ribosomally synthesized proteinaceous toxins that share a common biosynthetic pathway. Bacteriocins are generally narrow spectrum antibacterial agents with biological activity against closely related species and their genes are localized on transposable elements, plasmids or on the chromosome of the producer's genome. Modes of action range from depolarization of the lipid bilayer membranes [5,6], disruption of cell wall synthesis [7,8], inhibition of protein synthesis or degradation of host nucleic acids [9]. Mechanisms of cellular import are dependent on the target organism; enzymatic colicins that cross two lipid bilayer membranes and the peptidoglycan layer of the periplasmic space have a very different navigation pathway than lantibiotics such as mersacidin, which disrupt cell wall synthesis due to interactions with lipid II on the outside of the cell wall. The scope of this review is to investigate aspects of bacteriocin import. In particular, focus will be on the import processes adopted by colicins (especially enzymatic colicins), which have recently shown some exciting and novel mechanisms associated with cellular uptake [10–13].

Abbreviations: BRP, bacteriocin release protein; C domain, Cytotoxic domain; Col, colicin; IM, inner membrane; IUTD, intrinsically unstructured translocation domain; OM, outer membrane; OMP, outer membrane protein; PMF, proton motive force; R domain, Receptor binding domain; T domain, Translocation domain

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2. Colicin architecture

2.1. Domain organization and role in uptake

Colicins are modular proteins with three functional domains that act collectively to bind to the outside of a sensitive cell, cross the OM and translocate to a cytotoxic site of action (Fig. 1). Approximately 30–50% of the polypeptide is made up of a central receptor binding domain which interacts with a β-barrel shaped protein in the OM that is normally involved with the import of nutrients and metal ions such as vitamins, sugars and Fe^{3+}. An N-terminal translocation domain crosses the OM, in most cases through interactions with a second outer membrane protein that acts as a translocator and helps to establish a translocon through interactions with proteins termed Tol (mutations in which render the cell tolerant of the colicin) or Ton in the host periplasm. The translocator provides access for the colicin T domain to an energized translocation system in the periplasm. The T domain is divided into two parts; an intrinsically unstructured T domain (IUTD) and a larger structured T domain (STD) that is distal to the IUTD (Fig. 1). The final destination of the colicin molecule reflects the mode of action of the colicin which is either degradation of host nucleases in the cytoplasm (enzymatic colicins), depolarization of the cytoplasmic membrane (pore-forming colicins) or inhibition of peptidoglycan synthesis in the periplasm (colicin M) [14]. Based on this information, colicins are classified according to (i) the outer membrane receptor to which they bind and (ii) by the mechanism of translocation through the host periplasm according to Table 1. Import of Group A colicins occurs through the Tol-dependent translocation system which consists of the proteins TolA, TolB, TolQ, TolR and the peptidoglycan associated lipoprotein (Pal) whereas import of Group B colicins occurs through the TonB-dependent translocation system consisting of proteins TonB, ExbB and ExbD. Translocons of Tol-dependent colicins generally consist of interactions of the colicin with BtuB, OmpF and the Tol–Pal system, whereas translocons of the Ton-dependent system involve interactions of colicin with Ton and either FepA, FhuA or Cir. The primary receptor Tsx transcends the boundaries between Tol and Ton as it acts as primary receptor for the Tol-dependent colicin K and the Ton-dependent colicins 5 and 10 (Table 1). Interestingly, uptake of vitamin B12 which is TonB-dependent occurs from an initial interaction at the cell surface with BtuB whereas the BtuB-mediated uptake of colicins is exclusively associated with the Tol-dependent translocation of group A colicins rather than TonB (Table 1) [15].

2.2. Genetic arrangement of ORFs

Colicins are encoded on colicinogenic plasmids which are either low molecular weight, multi-copy plasmids of approximately 6 kb containing few other genetic determinants or high molecular weight, single copy plasmids in excess of 40 kb that carry many additional genes and are self-transmissible by conjugation [16]. The genetic organization of colicins varies depending on the type of colicin. The operon of enzymatic colicins consists of three genes; the first open reading frame in the operon specifies the structural gene of the colicin (named *cxa* for *c*olicin *x a*ctivity). *cxa* is immediately followed by a gene for immunity (*cxi*, named *c*olicin *x i*mmunity) which constitutively expresses a smaller polypeptide from its own promoter within *cxa* to neutralise the cytotoxic nuclease domain and prevent cell killing of the producing cell on synthesis of *cxa*. The third gene in the operon is termed *cxl* (named *c*olicin *x l*ysis), which is located distal to *cxi* and encodes a lysis protein that is involved in the extracellular release of colicins through lysis of the producer cell.

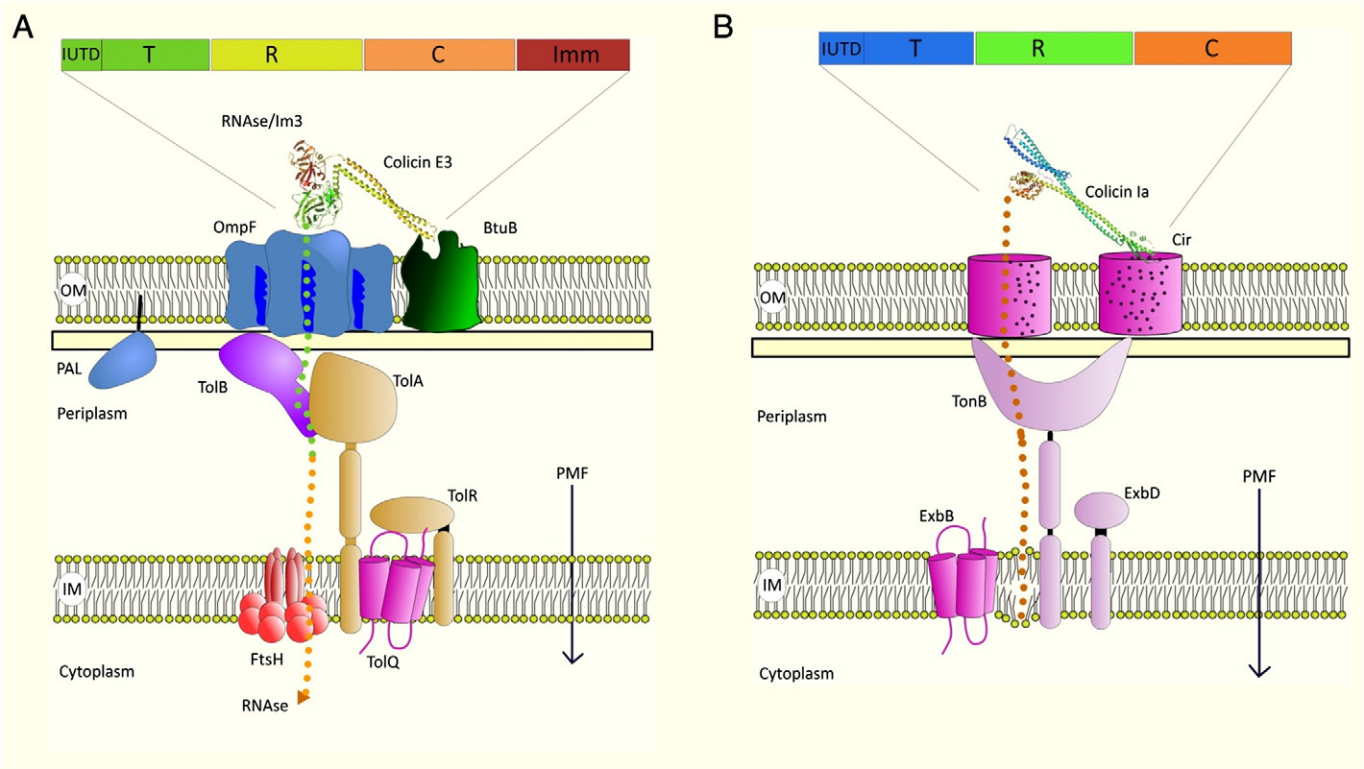

Fig. 1. Model of the colicin import mechanism. A. Enzymatic colicins such as colicin E3 bind to the outer membrane receptor, BtuB via their central receptor binding (R) domain at an angle of 45° from the cell surface. This permits the passage of the IUTD (green dots) through the lumen of an ompF monomer and across the OM to capture TolB. The resultant colicin translocon is energized by TolA, immunity is released from the colicin-immunity complex and the nuclease domain crosses the cell envelope (orange dots) by an as yet unknown mechanism. FtsH dependent processing of the colicin at the IM cleaves the nuclease domain from the colicin in the cytoplasm. In the absence of colicin, TolB forms an interaction with Pal at the membrane surface. B. Colicin Ia uses two copies of the outer membrane receptor, Cir; one for receptor binding and the 2nd one as a translocator. The Colla IUTD penetrates the cavity of the translocator (blue dots) following distortion of the Cir plug (black dots) to contact TonB at the boundary between OM and periplasm. Energy generated by the TonB–ExbB–ExbD complex promotes transport of the pore-forming domain into the IM (orange dashes). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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