

Two-Component Regulatory Systems – implication in the quorum sensing mechanisms and bacteriocin production in lactic acid bacteria

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ABSTRACT

For lactic acid bacteria, the mechanisms of quorum sensing and response are mediated by peptides or pheromones that interfere with the synthesis of antimicrobial peptides (AMP) called bacteriocins, when these molecules reach a certain critical level of concentration. Generally, the synthesis and activity of pheromones is adjusted by means of a two-component regulatory system. The observation that some microorganisms, in particular lactic acid bacteria, produce bacteriocins according to the cell density, has led to the discovery of the involvement of QS mechanisms in the synthesis of these peptides. Bacteriocins synthesis is inducible, the process requiring the extracellular accumulation of peptides that functions as chemical messengers activators of bacteriocins synthesis. This minireview presents the molecular architecture and functions of two-component regulatory systems and ABC transporters implicated in the synthesis and secretion of nisin, one of the most studied bacteriocin. The elucidation of the intimate mechanisms of bacteriocins synthesis is equally of biotechnological and medical importance, opening interesting perspectives for the development of improved technologies for the production of bacteriocins with good yields, and also, for increasing the beneficial anti-infective roles of probiotic bacteria when administered *in vivo*.

Keywords: antimicrobial peptides, nisin synthesis, probiotic, ABC transporters.

1. INTRODUCTION

Cell-cell communication is ubiquitous in the bacterial world and the understanding of this process is fundamental to all fields of applied microbiology, including microbial ecology, industrial and clinical microbiology [1]. The evolution of this process in bacteria could possibly have been driven by the need to sense the dynamics of their immediate environment, as opposed to the need for a concerted response. Since multiple factors could influence the direction of development in evolution, both theories could be correct and therefore have a right for coexistence [2].

To allow the efficient colonization and adapting to various environment conditions, the lactic acid bacteria require a sensory system for environment signals specific detection. generally, in bacteria, this function is mediated by a two-component regulatory system (TCS), which consists of a membrane-located histidine protein kinase (HPK) that monitors one or more environmental factors, and a cytoplasmic response regulator (RR), which

modulates expression of specific genes. HPK and RR work together as a signal transmission system which is based on a phosphorylation signal-transduction system [3] (figure 1). TCSs monitor and provide responses to the environment changes, like: osmolarity, available nutrients (C, N, P), temperature. In the same time, TCSs interacts with the specific molecules called pheromones which are implicated in the bacterial quorum sensing mechanisms [3, 4, 5].

For Gram positive bacteria, the TCSs-QS system is involved in the pathogenity and virulence genes expression or bacteriocins synthesis [6]. This coordination is achieved based on the cellular density by using peptide signalling molecules (autoinducers), these being frequently modified post-transcriptionally and exported through specific transport systems [7].

2. THE PROTEIC ARCHITECTURE OF TCS

The functionality of the two components system is based on successive phosphorylation processes by transferring the radical phosphate resulting from ATP hydrolysis to a protein receptor. Considering this, the two components of TCS have a functional domain organization and a classification system using structural characteristics [8]. Signal transduction systems show specific and/or dynamic localization, engaging in the spatial program of the bacterial cell [9].

The prototypical histidine kinase is a homodimeric integrative membrane protein that exhibits transmembranary (TM) helices (5-7 segments) with the extracellular N-terminus domain and an intracytoplasmic C-terminus domain implicated in binding the ATP to a histidine residue (the final acceptor of the phosphoryl group) [10]; this domain has a modular organization containing four homologous boxes: H, X, N, G: H box: histidine phosphorylation situs – contains 2 tyrosine (Tyr) residues and 1 proline (Pro) residue; X box: a hydrophobic region available for

some subfamilies; N box: contains only 1 preserved asparagine (Asp) residue; G box: has essential implications in the transmission of the phosphate group to the regulator protein. RR (response regulator) contains on its side one DNA binding C-

3. THE ABC – NIS T TRANSPORTER

The ABC (ATP Binding Cassette) type transporters are essential structures in the physiological mechanisms of any eukaryotic or prokaryotic living system, being involved in the transportation of various number of molecules, covering from ions, sugars, amino acids, antibiotics to different types of macromolecules as oligosaccharides, oligopeptides and proteins with high molecular mass [11].

They convert the energy gained from ATP hydrolysis into trans-bilayer movement of substrata either into the cytoplasm (import) or out of the cytoplasm (export) [12, 13]. ABC transporters are also involved in diverse cellular processes including maintenance of osmotic homeostasis, drug resistance, antigen processing, cell division, bacterial immunity, pathogenesis and sporulation [14, 15, 16, 17, 18, 19].

The first components identified by Neu in 1965 were substratum binding proteins (SPB) from the ABC transporters family, that are present in the periplasmic space of the Gram negative bacteria. For the Gram positive bacteria and Archaea, the proteins which bind the substratum are exposed on the cell surface and anchored to the cytoplasmatic membrane through some trans-membranary lipids and peptides, forming a molecular complex [20, 21]. For the lactic acid bacteria, the ABC transporters are involved in the pre-bacteriocin molecules transportation from the intracellular to the extracellular space [22].

The bacterial ABC exporter systems are functionally subdivided on the basis of the type of substrate that each of them translocates. We describe three main groups: protein exporters, peptide exporters, and systems that transport nonprotein substrata [23].

3.1. Structure

Based on their implication and origin, the ABC transporters have a structure organized in 4 domains: two highly hydrophobic trans-membrane domains and two ATP binding domains. The hydrophobic trans-membrane domains (TMD) are located at the N-terminus, consisting each of six trans-membrane helices which create a pore in the cell membrane. In the ABC system they are forming the attaching sites of the substrate-binding proteins and, subsequently, are sending the signal for binding the ATP molecule to the intra-cytoplasmatic domain of the nucleotides [24, 25]. ATP binding domains form a dimeric structure being located at the C-terminus, involved in the hydrolysis processes, providing the energy required for the conformational changes which induce the transportation of the molecules through membranes [14]. The structure of this domain consists in two subdomains, one with Rec-like structure and the other one with a helical structure [26]. The ATP binding situs is represented by two conserved sequences which are present in the structure of other types of proteins that bind ATP [27]. These are the reasons for binding the radical phosphate of ATP and ADP and orienting the Mg^{2+} in the binding situs. The glutamate from the second conserved sequence represents the hydrolysis catalytic situs, binding to the Mg^{2+} and to

terminus domain with the HTH (helix-turn-helix) structure and one N-terminus domain, which contains an aspartate residue that accepts a phosphoryl group from a cognate HPK [10].

the water molecule that performs the hydrolysis. Mutations of the rest of the glutamate are leading to the lost of ATP activity [28]. The first conserved sequence (with the Q loop structure) is generating hydrogen bounds with the H_2O molecule involved in the hydrolysis (together with the Mg^{2+} ions). On top of this, the H domain histidine residue supplementary generates more hydrogen bounds with ATP molecules [29].

3.2. Mechanism of transportation

The simplest model of the ABC transporters functionality operates in 4 steps [11]: 1- binding the substratum to the substratum-binding protein (SBP); 2- the binding protein is anchoring to the trans-membranary domain (TMD) and signals the cytoplasmic domain to bind ATP (NBD - Nucleotide Binding Domain); 3- after ATP binding, conformational changes of the transmembranary domain are induced, which triggers the extracellular exposure of a binding situs of the substratum and in this manner the substratum molecule is transferred to this domain; 4- afterwards, the ATP hydrolysis induces detachment of the nucleotide-binding domain dimers and orientate the substratum binding site from the cytoplasmatic domain to the internal membrane.

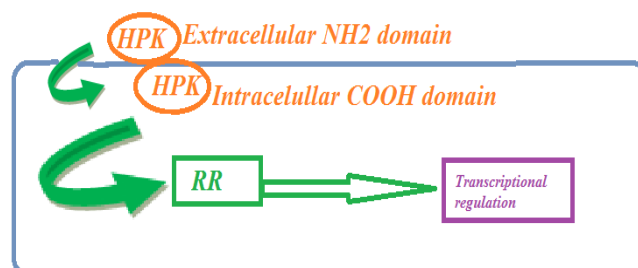


Figure 1. General signal transduction mechanism of the two-component regulatory systems. The cellular localization and response regulators (cytoplasmic) are represented.

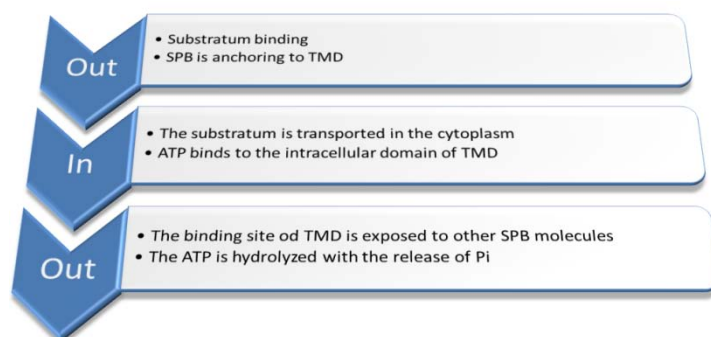


Figure 2. Functionality of the ABC transporters; SBP - substratum binding protein; TMD – Transmembranary Domain.

The substratum molecule is directly transferred in to the cytoplasm and SPB detach itself from TMD (fig. 2) [11, 30, 31].

The transfer of a molecule can be done in reverse (from the inside to the outside – efflux or secretion mechanism) without the

implication of the substrate binding proteins. In this case, the substrate molecule is attaching to the binding sites exposed on the internal face of the cytoplasmatic membrane, inducing the ATP

binding and conforming modification of the trans-membranary domain, the substratum molecule being secreted in the extra-cellular space.

4. CONCLUSIONS

Quorum sensing and response is an ubiquitous regulatory mechanism, involved in monitoring cell density of bacterial populations. Basically, the signal molecule (pheromone) is secreted at a low but constant rate by the most bacterial population cells. The pheromone concentration reflects the bacterial population cellular density during growth and, at a certain threshold concentration activates the pheromone-dependent two components regulatory system, having as consequence the inhibition or activation of various physiological processes. For lactic acid bacteria, the mechanisms of quorum sensing and response are

mediated by peptides or pheromones that interfere with the synthesis of antimicrobial peptides (AMP) called bacteriocins, when these molecules reach a certain critical level of concentration. The elucidation of the intimate mechanisms of bacteriocins synthesis is equally of biotechnological and medical importance, opening interesting perspectives for the development of improved technologies for the production of bacteriocins with good yields, and also, for increasing the beneficial anti-infective roles of probiotic bacteria when administered *in vivo*.

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