Two-component signaling pathways modulate drug resistance of *Staphylococcus aureus* (Review)

SHIZHOU WU[1,2*], KAIFENG LIN[2*], YUNJIE LIU[3], HUI ZHANG[1] and LEI LEI[2]

[1]Department of Orthopedics, West China Hospital, Sichuan University; [2]State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University; [3]West China School of Public Health, Sichuan University, Chengdu, Sichuan 610041, P.R. China

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Abstract. As the issues surrounding antibiotic-resistant strains of *Staphylococcus aureus* (*S. aureus*) are becoming increasingly serious concerns, it is imperative to investigate new therapeutic targets to successfully treat patients with *S. aureus* infections. The two-component signal transduction system is one of the primary pathways by which bacteria adapt to the external environment, and it serves an important role in regulating virulence gene expression, cell wall synthesis, biofilm formation and bacterial activity. There are 17 two-component signaling pathways in *S. aureus*, among which WalKR/VicSR/YycGF, AirSR/YhcSR, vancomycin resistance associated regulator/sensor and LytRS have been demonstrated to serve vital roles in regulating bacterial resistance, and are hypothesized to be potential targets for the treatment of *S. aureus* infections. The present review assesses the mechanism of the two-component signaling pathways associated with the development of *S. aureus* resistance.

Contents

Correspondence to: Dr Lei Lei, State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, 14 Renmin South Road, Chengdu, Sichuan 610041, P.R. China
E-mail: leilei0504@126.com

Professor Hui Zhang, Department of Orthopedics, West China Hospital, Sichuan University, 37 Guoxue Alley, Wuhou, Chengdu, Sichuan 610041, P.R. China
E-mail: caesarzh@163.com

*Contributed equally

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1. Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive coccus with low-GC content (1). Due to variations in certain genes in the bacterial genome and the introduction of exogenous genes, this strain exhibits different degrees of drug resistance against β-lactams, quinolones and aminoglycosides (1). Since osteomyelitis is caused by methicillin-resistant *S. aureus* (MRSA), investigating novel targets and methods for treating MRSA has become an urgent clinical need (2).

Bacteria need to regulate their metabolism in response to changes due to environmental stress, such as antibiotics, temperature and oligotrophy (3). Two-component signal transduction systems (TCSTs) are primary signaling mechanisms utilized by bacteria to regulate metabolism in response to environmental changes (3). The mechanisms underlying TCSTs regulatory systems are activated following physical or chemical stimuli from the external environment; the histidine protein kinase receptor proteins anchored to the cell membrane sense the change in the external environment and undergo phosphorylation. The phosphate group is transferred to the response regulator which undergoes phosphorylation, and this is mediated by phosphotransferase. Subsequently, the phosphorylated response regulators bind to the promoter sequences of the downstream target gene, thereby regulating its expression, upregulating expression of proteins which allow the bacteria to adapt to external environmental changes and enhance the adaptive viability of bacteria (4,5).

The two-component signaling pathways are widely found in prokaryotic bacteria, such as *S. aureus* (6). The entire genome of *S. aureus* has been analyzed and it has been demonstrated that there are 17 two-component signaling pathways involved in the regulation of bacterial physiological metabolism (7). *S. aureus* resistance is inextricably associated with the regulation of the two-component signaling pathway (8). The present

review assesses the current body of literature available regarding the mechanisms of two-component signaling pathways, and the emergency of developing suitable therapeutics to combat drug resistant *S. aureus* infections.

2. WalKR/VicSR/YycGF two-component signaling pathway

WalKR is a highly conserved and specific two-component signaling pathway in Gram-positive bacteria with low GC-content (9). *S. aureus* is Gram-positive with low GC-content (10). The *walKR* gene is an essential gene that is involved in the regulation of cell wall synthesis and other types of physiological metabolism (10). Howden *et al* (11) analyzed 10 types of vancomycin-intermediate resistance in *S. aureus* (VISA) and three types of heterogeneous VISA (hVISA) strains via high-throughput sequencing. The results demonstrated that eight types of VISA and two types of hVISA strains harbored site mutations in the *walKR* gene, and that this gene exhibited the highest degree of mutational frequency (11), suggesting that the *walKR* gene serves an important role in the generation of VISA and hVISA strains. The walKR protein not only participates in the regulation of cell wall synthesis, but also specifically binds to the promoter regions of the *lytM*, *atlA*, *ssaA* and *isaA* genes to positively regulate the expression of autolysin and peptidoglycan hydrolase, so as to modify the surface structure of the cell wall, including shortening the length of sugar chains in the cell wall and decreasing the degree of cross-linking of peptidoglycan (11). By changing the phenotype of the cell wall to increase bacterial resistance, the effectiveness of antibiotics which target the cell wall, such as vancomycin, is suppressed (11,12).

Vancomycin is a glycopeptide antibiotic secreted by *Streptomyces orientalis*, which inhibits cell wall synthesis by binding to the D-alanyl-D-alanine residue on the cell wall (13). In the absence of cell wall protection, the soma is prone to rupture, thus acting as a bactericide. A previous study showed that the insertion of the strong promoter sequence IS256, in the promoter region of the *walKR* gene of the *S. aureus* VISA strain upregulates the expression of its downstream target protein and enhances metabolic processes involving the strain's cell wall, resulting in an increase in bacterial resistance (14). In addition, single nucleotide polymorphisms are another important factor which underlie changes in WalKR expression. Domains in the *walKR* gene of the VISA strain carry a site mutation of a single nucleotide. The A96T mutation in the *walKR* gene is the mutation of base G into base A at site 24673 of *S. aureus*. The mutated base is located in a conserved sequence of the *walKR* gene and is associated with a conformational change of protein regulation by phosphorylation (15). These site mutations result in a decrease in the activity of the WalKR protein, downregulating the expression of bacterial autolytic enzymes, inhibiting autolytic processed in bacteria and decreasing the sensitivity of the bacteria to the drug (11).

The protein expressed by the *yycHI* gene as a downstream target gene of *WalKR*, binds to the WalKR histidine kinase receptor to form a complex to inhibit the activation of WalKR, and thus negatively regulates the WalKR pathway (16). The mutation rate of the *yycHI* gene in the VISA strain isolated from a clinical trial was significantly higher compared with that of the vancomycin-sensitive strain (16). This may due to the fact that the mutation of *yycHI* gene resulted in weakening of the negative regulatory mechanism on the WalKRK pathway, enhanced cell wall modification and cell wall synthesis and improved bacterial resistance (11).

3. AirSR/YhcSR two-component signaling pathway

The AirSR two-component signaling pathway is closely associated with *S. aureus* resistance towards vancomycin. In *S. aureus* (NCTC8325, a prototypical strain of *S. aureus* stored at National Collection of Type Cultures) with an *airSR* gene mutation, the expression levels of ~30 genes associated with cell wall synthesis, such as *cap*, *ddl* and *pbp1*, is significantly decreased and the minimum inhibitory concentration of the airSR gene mutant against vancomycin is significantly decreased. Further investigation using EMSA technology showed that the AirR protein directly binds to the promoter sequences of cell wall-forming genes such as *cap*, *ddl* and *pbp1*. By positively regulating the expression of these genes, the anabolic process of the cell wall was promoted, which in turn increased the sensitivity of the bacteria to vancomycin (17).

The YhcSR gene is an essential gene in *S. aureus* that is primarily involved in the regulation of important physiological processes, such as nitrate respiratory metabolism (18). The nitrate respiratory metabolic pathway is a key metabolic pathway required for the survival of *S. aureus* in a hypoxic environment (19). In addition, the YhcR protein directly binds to the promoter regions of the *opuC* and *lac* genes, and positively regulates the expression of these two genes. The *opuC* gene primarily participates in the synthesis of ABC transporters, whereas the *lac* gene is primarily involved in the metabolic regulation of lactose and galactose (20).

4. Vancomycin resistance associated regulator/sensor (VraRS) two-component signaling pathway

VraRS two-component signaling pathway is another important pathway associated with vancomycin resistance. The thickness of the *S. aureus* cell wall is positively correlated with the degree of vancomycin resistance (21). Proteins such as PBP2, SgtB and MurZ are key proteins involved in the synthesis of the cell wall of *S. aureus* and are positively regulated by the VraRS two-component signaling pathway (22,23). Kuroda *et al* (24) found that the decrease in vancomycin sensitivity was associated with the increased expression of the *vraRS* gene in the resistant *S. aureus* strain, and the authors showed that the expression levels of the *vraRS* gene was significantly increased by placing *S. aureus* in a medium containing vancomycin. Further investigations of several other antibiotics that act on *S. aureus* and inhibit cell wall synthesis, such as teicoplanin, ceftizoxime, imipenem, bacitracin and cycloserine, were performed, and it was shown that the expression levels of the *vraS* gene were increased (24). Based on these results, it was hypothesized that when *S. aureus* is stimulated by cell wall synthesis inhibitors, the cell wall synthesis process is positively regulated through upregulating expression of the *vraRS* gene, thereby reducing the sensitivity to the antibiotics that inhibit cell wall synthesis.

5. LytRS two-component signaling pathway

Cationic antimicrobial peptides (CAPs) are a class of active peptides produced by host cells, which are involved in the

body's innate immune system (25). CAPs bind to the bacterial cell membrane through electrostatic attraction. Due to the oil-water amphipathy, the small molecule active peptide is inserted into the cell membrane of bacteria, and destroy the integrity and increase the permeability of the cell membrane, leading to the lysis and death of cells (25). When the surface potential of the bacterial cell membrane is decreased by CAPs, the LytS receptor in the LytRS two-component signal undergoes autophosphorylation and activates the corresponding response regulatory protein, LytR. The LytR protein binds to the corresponding promoter regions of the downstream target genes, such as the *irgAB* gene (26). The *irgAB* gene is a downstream gene adjacent to the *lytRS* gene and is crucially involved in the synthesis of anti-perforin, which inhibits programmed cell death and lysis (27). Thus, the LytRS two-component signal regulates the upregulation of the *irgAB* gene, inhibits programmed cell death and autolytic enzyme activity of bacteria, and therefore enhances the drug resistance of bacteria.

Furthermore, the *lytRS* gene serves a role in regulating bacterial extracellular DNA, which is an important component of biofilms (28). When the surface potential of the bacterial cell membrane is decreased, the activation of this pathway subsequently decreases the secretion of extracellular DNA and inhibits the formation of biofilms (26). As the formation of the bacterial membrane improves bacterial resistance (29), the *lytRS* gene may decrease bacterial resistance by negatively regulating the secretion of extracellular DNA. Thus, the LytRS two-component signaling pathway may exhibit dual-regulation in the resistance of *S. aureus*. The *yhcSR* and *vraRS* genes in *S. aureus* are homologous to the *yhcYZ* and *yvqEC* genes in *Bacteroides subtilis* (*B. subtilis*). The *yhcYZ* and *yvqEC* genes in *B. subtilis* interact and regulate cell wall synthesis (30). Thus, it is hypothesized that *yhcSR* and *vraRS* genes may also employ similar mechanisms, namely, regulating cell wall synthesis.

6. GraRS/ApsRS two-component signaling pathway

Bacteriocin is a peptide substance that is self-synthesized by bacteria to inhibit the proliferation of other types of bacteria. These peptides are released into the cytoplasm with the assistance of the ATP-binding cassette (ABC) transport system (31). In addition to releasing its own bacteriocin, the ATP transport system also pumps exogenous bacteriocins out of the cytoplasm as a defense mechanism (32). Multiple ATP gene loci adjacent to the bicomponent signal were identified following the analysis of the gene locus of *S. aureus* (32). However, *S. aureus* has not been confirmed to possess bacteriocin synthesis-associated genes, and therefore these structural sites are hypothesized to be primarily associated with immune defense (33). High expression of genes associated with the two-component signaling pathway in GraRS is associated with VISA production (34). The GraRS two-component signaling pathway upregulates the expression of VraFG ATP transporter-associated genes, enhances efflux transport mechanisms in bacteria, increases transport of intracellular antibiotics, such as vancomycin and mitomycin B, out of the cytoplasm and prevents antibiotics from exerting antibacterial effects (35).

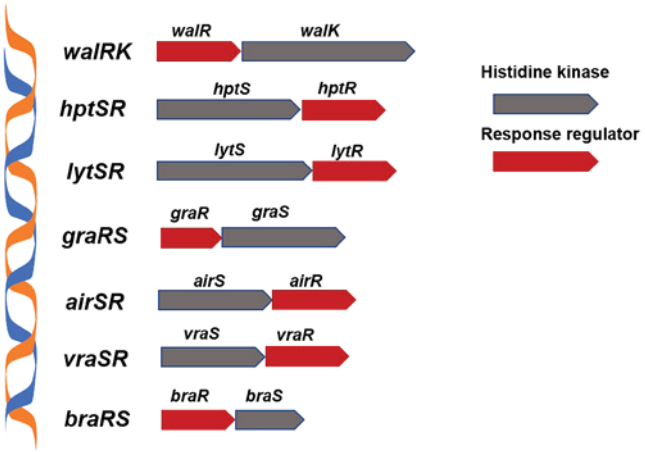

Figure 1. Two-component signaling pathways in *S. aureus*. Representation of the *S. aureus* genome with the seven operons encoding two-component systems. Histidine kinases are colored red, and response regulators are colored gray. *S. aureus*, *Staphylococcus aureus*; R, response regulator; K, kinase; S, sensor; VraRS, Vancomycin resistance associated regulator/sensor.

Conversely, the *dlt* and *mprF* genes decrease the negative charge on the surface of the cell membrane through modifying components, such as teichoic acid and phosphatidylglycerol on the surface of the cell membrane (36,37). The *GraRS* gene regulates the expression of *dlt* and *mprF* genes, and alters the negative potential on the cell wall surface, thereby decreasing the binding capacity of positively charged antibiotics (such as vancomycin and mitomycin B), and weakens their antibacterial effect (35). However, currently, the mechanism underlying the activation of the GraRS receptor protein is unknown, and the mechanism underlying the effect of the GraRS pathway on drug resistance requires further investigation.

7. BceRS/BraRS/NsaRS two-component signaling pathway

Similar to the GraRS/ApsRS two-component signaling pathway, the BceRS two-component signaling pathway gene is adjacent to the *bceAB* and *vraDE* genes of the ABC transport system (38). *bceAB* is located upstream of the *bceRS* gene, whereas vraDE is located ~80 bases downstream of the *bceRS* gene (38). The BceS protein activates the BceR protein following stimulation by external bacteriocin, and this results in the upregulation of the expression of *bceAB* and *vraDE* genes, thereby increasing transport of exogenous bacteriocins out of the cytoplasm by BceAB and VraDE proteins, preventing bacteriocin from acting as an antibacterial agent, and thus resulting in an increase in bacterial resistance (39). The minimum inhibitory concentration of *S. aureus* towards bacteriocin is decreased by 2-4-fold by reducing *bceAB* and *vraDE* gene expression (which encode the APC transporter gene) in *S. aureus* (38).

Nisin A. and Nukacin ISK-1 are type I bacteriocins secreted by *Staphylococcus warner* and *Lactococcus lactis*, respectively (40,41). Nisin A. exerts its bacteriostatic effects by acting on the cell membrane of bacteria to form pore complexes, which cause leakage and dissolution of cell fluid. Additionally, Nisin A also serves a role in inhibiting cell wall synthesis (40). Nukacin ISK-1 serves an antimicrobial role primarily via inhibiting the synthesis of the cell wall (41).

When the two bacteriocins were co-cultured with the *BraRS* gene mutant *S. aureus*, the growth of the mutant strain was significantly inhibited (50-33%) (42). Thus, the BraRS gene exhibits significant regulatory effects on the symbiosis of *S. aureus* and the type I bacteriocin strain.

8. Hexose phosphate transporter regulator/sensor (HptRS) two-component signaling pathway

The HptRS two-component signaling pathway is primarily composed of *hptA*, *hptRS* and *uhpT*. HptA initiates autophosphorylation of the HptS protein by sensing changes in the concentration of surrounding phosphates, such as 3-phosphoric acid glucose, glucose-6-phosphate and Fosfomycin. *uhpT* is a downstream regulatory gene of the HptRS two-component signaling pathway. UhpT protein transports the aforementioned phosphates into the cytoplasm of bacteria to provide a source of carbon for physiological metabolism in bacteria (43). In addition to extracellular growth, *S. aureus* may invade host epithelial cells and acquire phosphate hexose from the cytoplasm of the host cell to maintain physiological metabolism in the bacteria (44) via the phosphoenolpyruvate phosphotransferase pathway (45). The molecular structure of Fosfomycin, a broad-spectrum antibiotic in clinical trials, is similar to phosphoenolpyruvate. Instead of being metabolized by the body, the antibiotic is excreted in its original form, thus it is widely used for the treatment of osteomyelitis due to its lower toxicity and fewer side effects (46). This type of antibiotic may be transported into the cytoplasm via the bacterial phosphohexose transporter to exert its antibiotic effects through competitively binding to UDP-N-acetylglucosamine-3-O-enolpyruvate transferase (encoded by the *murA* gene), inhibiting the synthesis of the peptidoglycan precursor, as well as interfering with the synthesis of the cell wall (47). However, when mutations occur in the HptPS two-component signaling pathway, the expression of UhpT protein is decreased, accompanied by a decrease in the uptake of Fosfomycin, and thus a decrease in the effectiveness of the drug, resulting in enhanced bacterial resistance (43).

9. Conclusion

As the incidence of MRSA infections is increasing each year, the therapeutic effects of antibiotics are becoming notably decreased. Although the emergence of novel treatment methods face enormous challenges, research based on new treatment concepts is required to combat emergent resistant MRSA strains. The two-component signaling pathway of *S. aureus* (Fig. 1) regulates the sensitivity of strains to antimicrobial agents through efflux mechanisms, regulation of cell wall anabolic processes, and inhibition of drug uptake. Furthermore, the two-component signaling pathways may regulate bacterial physiological metabolism and virulence factors, and improve the adaptability of bacteria to the external environment. Hence, an in-depth study of the mechanisms involved in the two-component signaling pathway of *S. aureus* may highlight novel potential targets for the treatment of osteomyelitis.

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Availability of data and materials

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Authors' contributions

SW, KL, YL, HZ and LL conceived and designed the present review. SW, KL, YL, HZ and LL drafted and critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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