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RecA: a universal drug target in pathogenic bacteria

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1. ABSTRACT

The spread of bacterial infectious diseases due to the development of resistance to antibiotic drugs in pathogenic bacteria is an emerging global concern. Therefore, the efficacious management and prevention of bacterial infections are major public health challenges. RecA is a pleiotropic recombinase protein that has been demonstrated to be implicated strongly in the bacterial drug resistance, survival and pathogenicity. In this minireview, RecA's role in the development of antibiotic resistance and its potential as an antimicrobial drug target are discussed.

2. INTRODUCTION

Antimicrobial chemotherapy is mainly applied for the treatment of bacterial infectious diseases. The commercially available antibiotic drugs are broadly classified on the basis of the cellular component or cellular process they affect (1). In particular, genotoxic antibiotics such as fluoroquinolones (e.g., ciprofloxacin) target the essential for DNA replication enzymes DNA gyrase and DNA topoisomerase IV, leading to DNA breakage or stalled replication forks. Non-genotoxic antibiotics such as β-lactams (e.g., penicillins and cephalosporins), a class of antibiotics interfering with cell wall synthesis (2), and aminoglycosides (including streptomycin and kanamycin), which inhibit protein synthesis by binding to the 30S subunit of the bacterial ribosome (3), trigger the production of DNA-damaging reactive oxygen species (ROS) (4). However, pathogenic bacteria display a global decreasing susceptibility and increasing tolerance towards all known classes of antibiotics, leading to the emergence of untreatable infectious diseases (5). These pathogens are also referred to as "superbugs" (e.g., *Neisseria gonorrhoeae* strains H041) (6), as they demonstrate multidrug resistance. As a result, the evolution of antibiotic resistance constitutes a serious problem in the treatment of bacterial infections with financial impacts, since pharmaceutical companies spend millions for the discovery of novel antibacterial drugs (7). Therefore, the development of bactericidal drugs efficacious for the treatment of bacterial infections is of primary concern (8). Herein, the role of the bacterial recombinase RecA as a potential and effective drug target is discussed.

3. SOS RESPONSE

Antibiotic-induced stress has been shown to trigger the bacterial DNA damage response (SOS response) system (9), which is mainly controlled by RecA, a protein involved in the homologous recombinational DNA repair by catalyzing the DNA strand exchange of reaction in bacteria (10), and the repressor protein LexA (11, 12). Under normal conditions, the LexA dimer binds to a 20 bp consensus palindromic DNA sequence (SOS box), leading to the transcriptional repression of a SOS regulon harboring an ensemble of over 50 genes. The SOS response genes are induced in a temporal fashion, whereby the genes involved in nucleotide excision repair (*uvrABC*) or recombination (*recA*) are induced first, while genes encoding the error-prone DNA polymerases Pol II (*polB*), Pol IV (*dinB*) and Pol V (*umuC* and *umuD*) are induced in the late stages of SOS response repair. Under antimicrobial stress, RecA is activated by polymerizing around exposed single-stranded DNA (ssDNA) to form a nucleoprotein filament (RecA*) in an

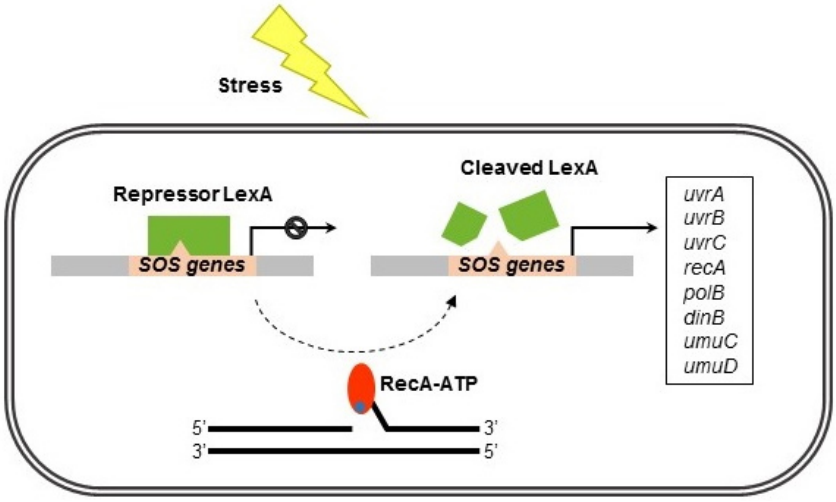

Figure 1. The SOS response system. Antibiotic-induced stress leads to RecA activation which stimulates the self-cleavage of the repressor LexA and the subsequent activation of the SOS genes.

ATP-dependent manner. RecA* co-protease activity promotes the autocatalytic cleavage of LexA, thereby alleviating the repression of the SOS regulon (9–12) (Figure 1).

3.1.Adaptive drug-resistant mutations

The three low fidelity DNA polymerases, Pol II, Pol IV and Pol V, allow translesion DNA replication, introducing in this way mutations into the bacterial genome (13). In case these mutations take place in DNA regions that code for antibiotic target proteins, then mutated proteins with altered structure, and hence function, are generated upon which antibiotics cannot bind; as a result, the mutant bacteria eventually survive. For example, a point mutation in the *Staphylococcus aureus pbpB* gene coding for PBP2 (penicillin-binding protein 2) resulted in decreased susceptibility to a β-lactam antibiotic (14). In a recent study, mutations in the genes *gyrA* and *parE*, encoding DNA gyrase and DNA topoisomerase IV, respectively, were found in an antibiotic resistant *Pseudomonas aeruginosa* strain (15).

Therefore, the increased rate of accumulating adaptive mutations renders mutant bacteria resistant to a broad spectrum of antibiotics and, furthermore, facilitates the process of natural selection, favoring in this way the survival and multiplication of mutant bacteria.

3.2. Horizontal transfer of drug-resistance genes

Mobile genetic elements play a central role in the acquisition and dissemination of antibiotic resistance genes among pathogenic bacteria. These elements are transferred through conjugation and integrate into the genome of the host bacterium (16, 17). SOS response significantly stimulates the transfer of integrating genetic elements (ICEs), such as the *Vibrio cholera* SXT which contains genes that confer resistance to various antibiotics (18). It was shown that RecA co-protease activity was required for the transcriptional derepression of genes essential for SXT transfer by stimulating the autodegradation of the SetR repressor (18).

Integrons are mobile elements that encompass antibiotic resistance genes embedded in cassettes. The integron integrase gene (*intI*), required for the incorporation of exogenous gene cassettes and the recombination of endogenous gene cassettes, is up-regulated by SOS response (19, 20). Guerin *et al.* showed that RecA function was necessary to induce *intI* expression and hence gene cassette recombination (19).

The DfrB4 protein, encoded by the integron-related *dfrB4* gene, was identified in antibiotic-resistant *Escherichia coli* clinical isolates. DfrB4 conferred a markedly increased resistance to the antibiotic trimethoprim *in vitro*, leading to the suggestion that dfrB4 contributes to clinical antibiotic resistance (21).

4. POTENTIAL RECA TARGETS

Suppression or attenuation of the SOS response system via preferential inhibition of RecA is proposed as a possible therapeutic strategy to suppress the development of antibiotic multiresistance and resistance-conferring mutagenesis (22, 23). For example, deletion of *recA* in *E. coli* and *Staphylococcus aureus* resulted to a significant antibiotic-induced resistance reduction (24). Of note, the compound suramin effectively and selectively inhibited the recombinational and co-protease activity

of the *Mycobacterium tuberculosis* RecA protein. This compound also suppressed antibiotic-induced *recA* gene expression. However, the inhibitory potency of suranim on Rad51, the human structural and functional homologue of the bacterial RecA, has not been investigated (25).

Several site-directed mutagenesis studies have been performed to reveal specific amino acid mutations affecting specific structural and functional aspects of the RecA protein (Figure 2). In particular, Adikesaven and coworkes (26) have demonstrated that the amino acids E123, G165, A168, S172, R176, G212 clustered on the extended RecA-RecA/DNA interface are important for RecA-DNA nucleoprotein filament formation. Mutations in these residues severely impeded RecA recombinase activity and RecA's ability to mediate LexA autoproteolysis upon UV-induced DNA damage (26).

Moreover, the *Mycobacterium smegmatis* RecA residues Q196, R198 and F219 (the corresponding Q194, R196 and F217 in *E. coli* (Figure 2)), were essential for the RecA-DNA nucleoprotein filament formation (27).

Mutations of the amino acid E96 (E96D), essential in ATP hydrolysis throughout the RecA-mediated recombination process, as well as the residues H97, S117, I128, N249, K250, F255, located at the RecA subunit interface, were found to cause defective chromosome segregation and cellular toxicity *in E. coli* even in the absence of SOS response induction (28). Of note, the substitution of Glu96 by the fellow Asp acidic amino acid could not be tolerated and had detrimental effects to the host cell, although these two residues have virtually identical physicochemical properties (29).

The neighboring amino acid residues D224 and R226 located at the cleft region between the two RecA subunits are proposed to be required for RecA-RecA homodimerization. The D224A and R226A variants significantly impaired RecA-mediated recombinational activity and initiation of LexA self-degradation in *E. coli* (26). However, the residue D224, although invariant across bacterial species, is substituted by the fellow acidic glutamic acid E224 in *Bacillus anthracis* and *Staphylococcus aureus* (Figure 2). The effect of this amino acid variant in the RecA protein tertiary structure and RecA-mediated processes in the corresponding host organisms should be investigated. Moreover, mutations in the amino acids H97A and K248A, located at the interface of the two adjacent RecA monomers, led to defective RecA-mediated recombinational activities (30).

Combined disruption of the G22 and G108 residues, located at the amino- and carboxy-terminal domain of the RecA protein, respectively, markedly impaired RecA-LexA interaction and induction of LexA self-cleavage (26). These two amino acids, although distant from one another, they are found on the opposing complementary sides of the adjacent RecA monomers and they can therefore interact.

Pol V, which is principally responsible for in SOS-induced mutagenesis (31, 32), is composed of a mutagenically active UmuD' homodimer and a UmuC monomer (UmuD'$_2$C) (33, 34). RecA protein activates Pol V (35, 36) and mediates UmuD self-cleavage yielding UmuD' (37). The amino acid S117 was shown to interact with Pol V through its UmuC subunit and to be essential for its activation (38, 39). Moreover, the G204S mutant was shown to significantly suppress LexA and UmuD autocatalytic cleavage (26, 40). Of note, a relatively high concentration of the mutagenic UmuD'2C protein complex was demonstrated to inhibit RecA recombinase activity (41). Mutations in the L114 and S117 residues were shown to confer resistance to the suppression of RecA function imposed by UmuD' and UmuC overexpression (38).

The fact that the above amino acid residues are evolutionarily conserved across taxonomically diverse bacterial species (Figure 2) signifies their important role in maintaining the structural integrity of RecA protein and hence its functionality. These amino acid sites could be exploited in protein structure-based drug design for the identification of potent compounds that specifically block RecA protein's function in a wide range of pathogenic bacteria. Given that the pathogenic bacteria under study infect humans and other animals, it is important to ensure that cross-reaction with the infected host is avoided. To this end, the inhibitory effect of these compounds should be also tested on the Rad51 protein, the amino acid sequence and tertiary structure of which are highly conserved across all mammalian species.

5. CONCLUDING REMARKS

The pivotal role of RecA in acquired antibiotic-mediated mutagenesis, multidrug resistance and survival in pathogenic bacteria possessing the SOS response system point clearly into the direction that the most effective therapeutic strategy to both decrease the evolution of antibiotic resistance and attenuate bacterial pathogenicity would be the selective inhibition of RecA. Therefore, future antibiotic drug design efforts should be directed towards the development of a multipotent antibiotic that could target a wide range of pathogenic bacteria. Co-treatment with a drug selectively inhibiting RecA and a conventional antibiotic drug, such as rifamycins, might enhance the effectiveness and efficacy of chemotherapy.

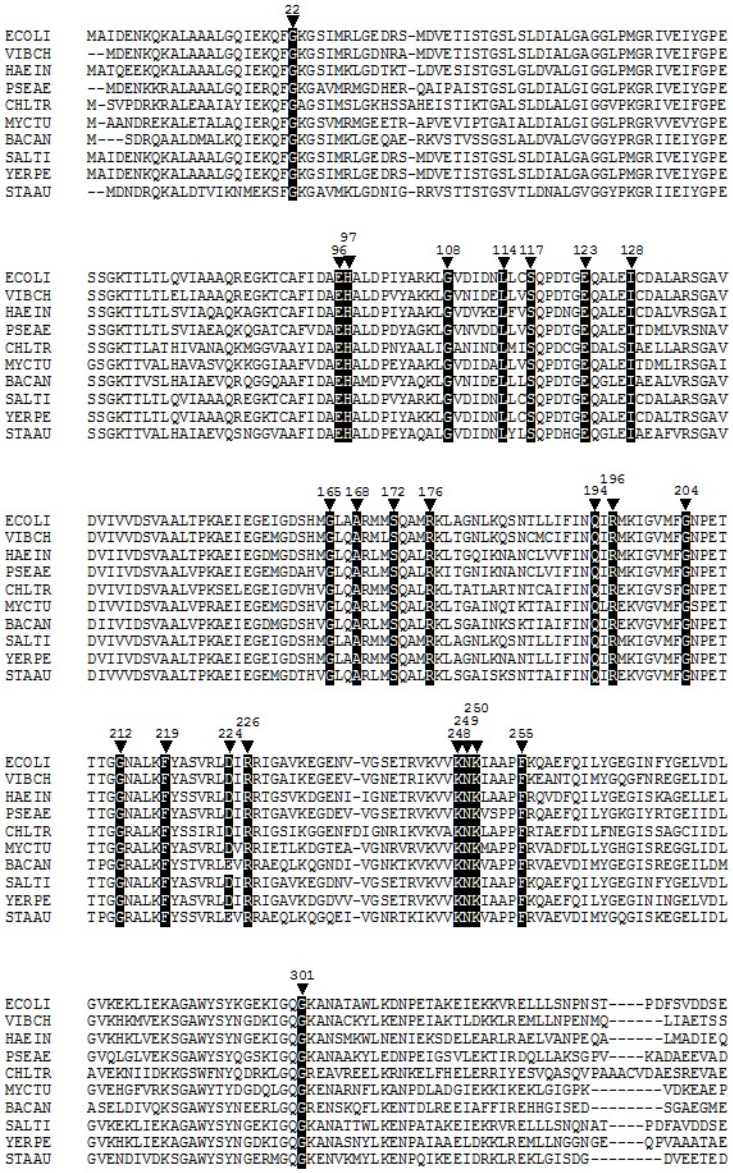

Figure 2. Alignment of the RecA protein sequences of 10 pathogenic bacteria possessing the SOS response system using PROMALS3D (42). The species names are indicated by the UniProt (43) 5 digit alphanumeric identification code and the corresponding UniProt accession code is shown within parentheses. ECOLI: *Escherichia coli* (P0A7G6); VIBCH: *Vibrio cholerae* (P45383); HAEIN: *Haemophilus influenzae* (P43705); PSEAE: *Pseudomonas aeruginosa* (P08280); CHLTR: *Chlamydia trachomatis* (P0CD80); MYCTU: *Mycobacterium tuberculosis* (A0A0T9KQ18); BACAN: *Bacillus anthracis* (Q9APZ2); SALTI: *Salmonella typhi* (P65978); YERPE: *Yersinia pestis* (P37858); RECA: *Staphylococcus aureus* (P68845). The evolutionarily conserved amino acid residues (arrowheads) are number according to the *E. coli* RecA (PDB: 3cmw). The invariant residues are depicted as white letters on black background and the conserved residues with similar physicochemical properties are shown in bold letters.

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