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recA gene expression in a streptomycete is mediated by the unusual C-terminus of RecA protein

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Abstract

Streptomyces RecA proteins are characterized by a conserved, positively charged extension of unknown function appended at their C-termini. To investigate the function of this element, we introduced the *Streptomyces rimosus recA* gene and its mutant form encoding the protein with a C-terminal deletion into *S. rimosus*. Both transcript and protein levels were dramatically increased in the strain expressing the truncated gene compared to the strain bearing the wild-type *recA*, indicating involvement of the characteristic C-terminal extension in regulating the *recA* expression in *Streptomyces*. Considering that RecA acts as a major regulator of DNA damage response in bacteria, this mode of regulation is expected to have broader implications and significance that outreaches our current understanding of RecA autoregulation.

Keywords: RecA; *Streptomyces*; C-terminus; Transcription; DNA damage response; Promoter

1. Introduction

RecA is a multifunctional protein that plays a central role in the process of homologous recombination, recombinational DNA repair and regulation of the DNA damage response [1]. It is amongst the most conserved bacterial proteins with an overall similarity between 43% and 100% [2], and is universally distributed within the bacterial kingdom, with the exception of a few obligate endosymbionts [3]. The most conserved part of RecA is its central domain that binds ATP and DNA, while its N- and C-terminal regions, involved in monomer interaction [4], display species-specific variety.

The role of RecA protein in regulating DNA damage response has been well established for the *Escherchia coli* system: it acts as an indirect regulator of a number of genes, including the *recA* itself, that are normally repressed by the LexA protein. The RecA nucleates onto single-stranded DNA produced as a result of DNA damage, forming an activated RecA filament. The filament in turn interacts with the LexA protein and stimulates its autocatalytic cleavage, thereby inducing the genes of the SOS-regulon [5]. In particular, binding of the RecA to both DNA and protein regulators is mediated by the negatively charged C-terminus of the RecA protein [6,7]. Consequently, this structural element is an important determinant of cellular RecA quantity control: it was demonstrated that removal of 25 residues from C-terminus increased levels of the RecA production and led to constitutive SOS induction [6].

Whereas *E. coli* and majority of bacterial RecA proteins possess shorter and acidic C-termini, a characteristic ~20 amino acid extension rich in alanines and lysines

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is present at the C-termini of Streptomyces RecAs [8,9] (Fig. 1A). The function and structure of this appendix are unknown, but deletion of this region in the *Streptomyces lividans* RecA has been shown not to affect major RecA functions (judged by the ability of truncated protein to restore UV-resistance and homologous recombination in a RecA-deficient strain) [10]; the same was confirmed for the C-terminal deletion in the *Streptomyces rimosus* RecA [11]. Moreover, our understanding of DNA damage response in *Streptomyces* is obscure: although the upstream regions of the streptomycete *recA* reveal the presence of a putative *recA* promoter overlapped by an imperfect LexA-binding site [12], transcriptional analysis of the *S. rimosus recA* showed that this putative promoter has only a very weak activity and is not significantly induced upon DNA damage [13] (Fig. 2). Instead, the major, DNA damage inducible *recA* promoter was identified 94 bp downstream (Fig. 2) as a novel type of promoter [13]. It has been experimentally demonstrated for the homologous *Mycobacterium*

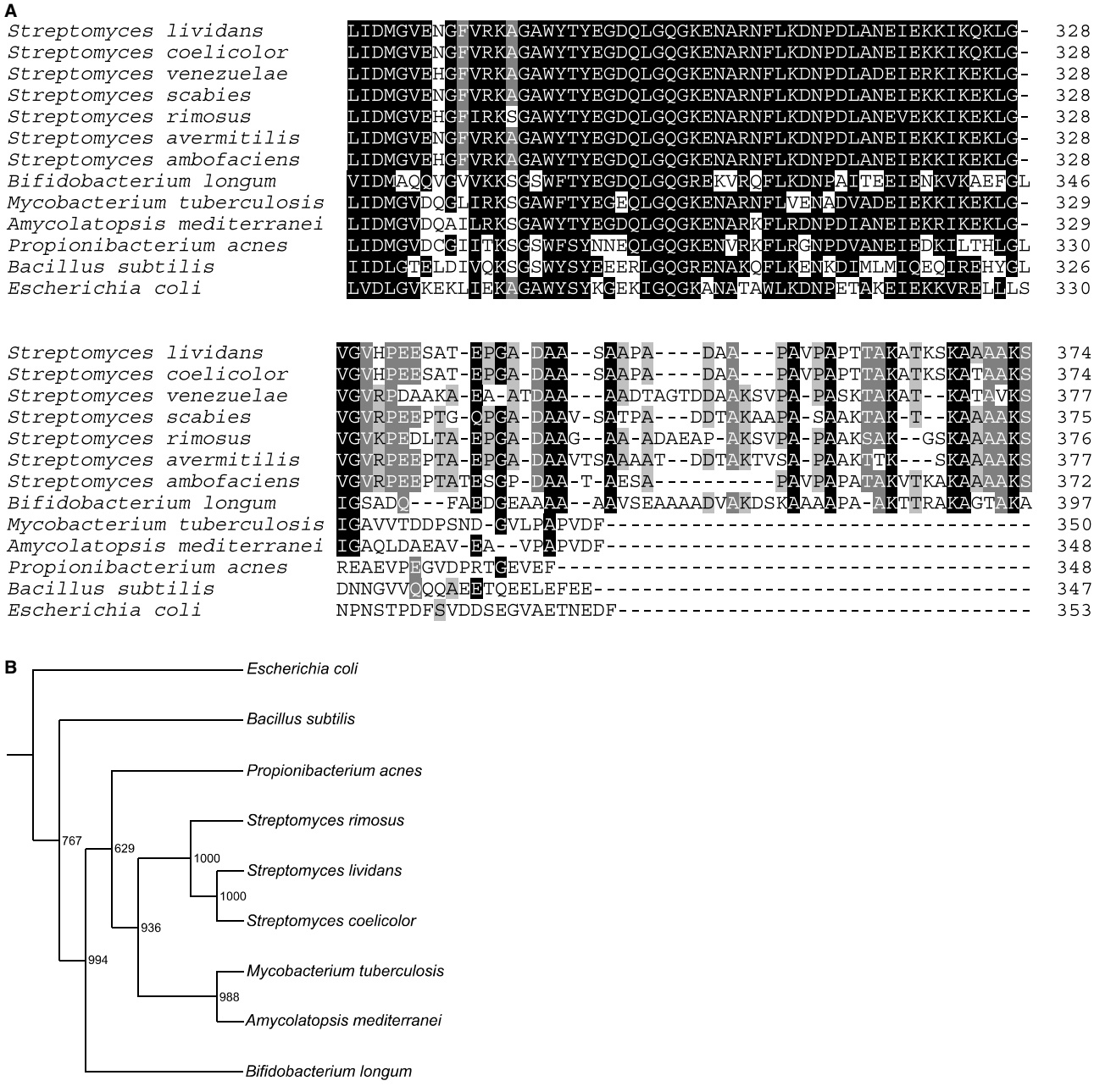

Fig. 1. (A) Multiple sequence alignment of the C-terminal regions of RecA proteins. GenBank accession numbers are as follows: *S. lividans* (P48294), *S. coelicolor* (O50487), *S. venezuelae* (U04837), *S. scabies* (http://www.sanger.ac.uk/Projects/S_scabies), *S. rimosus* (P95846), *S. avermitilis* (Q82KB1), *S. ambofaciens* (P41054), *B. longum* (AAN25214), *M. tuberculosis* (P35901), *A. mediterranei* (Q9REV6), *P. acnes* (AAT69987), *B. subtilis* (P16971) and *E. coli* (P03017). (B) Phylogenetic tree of selected bacterial RecA proteins. Bootstrap values are given for each branch. The tree has been rooted using archaeal RadA sequences.