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MicroCorrespondence

Essential monomer–monomer contacts define the minimal length for the N-terminus of RecA protein

Sir,

The RecA protein of *Escherichia coli* is critically involved in the process of homologous recombination and is the prototypic member of a large family of proteins found ubiquitously. The crystal structure of *E. coli* RecA protein shows that amino acids 3–21 at the N-terminus are organized in an α-helix, so that amino acid residues 6, 9, 10, 13, 14, 17, 20 and 21 are exposed on a surface involved in the interaction with adjacent monomers within the RecA nucleoprotein filament (Story *et al.*, 1992, *Nature* **355**: 318–325). These monomer–monomer interactions are crucial to the filamentous structure of the RecA nucleoprotein complex and, consequently, for all of its biochemical activities. In agreement, deletion of the first N-terminal 33 amino acids of RecA protein abolishes its ssDNA binding and ATP hydrolysis activities (Mikawa *et al.*, 1995, *J Mol Biol* **250**: 471–483).

We were specifically interested in knowing how formation of the RecA filament is affected by attenuation of the strength of monomer–monomer interactions. To investigate this, we constructed several N-terminal truncations of *E. coli* RecA protein that gradually decreased the number of amino acid contacts involved in monomer–monomer interactions. As shown in Table 1, all deletion derivatives that lost amino acid residues that are involved in monomer–monomer interactions (shown in bold font) manifest a RecA$^-$ phenotype. K6, the first amino acid residue involved in monomer–monomer interaction, is conserved in proteobacteria (substituted by an arginine in Gram positive) and forms a salt bridge with D139 of another monomer (Karlin and Brocchieri, 1996, *J Bacteriol* **178**:1881–94). The substitution K6A, which disrupts this electrostatic interaction, but which retains hydrophobic interaction, only weakly sensitizes cells to UV light (Morimatsu and Horii, 1995, *Adv Biophys* **31**: 23–48), suggesting that the K6–D139 salt bridge is not essential for RecA protein function. However, comparison of RecA Δ9 with RecA wild type and considering only bold-faced residues in Table 1 suggests that K6 (or other amino acids at this position capable of hydrophobic interactions) is required for monomer–monomer interactions. Our data therefore suggest that the loss of only the first monomer–monomer contact (K6) is sufficient to reduce the strength of monomer–monomer interactions below that required for the RecA nucleoprotein filament formation and function.

This conclusion allowed us to uncover a mistake propagated in all published alignments of bacterial RecA protein sequences (Roca and Cox, 1990, *Crit Rev Biochem Mol Biol* **25**:415–456; Karlin and Brocchieri, 1996, *J Bacteriol* **178**:1881–94; Roca and Cox, 1997, *Prog Nucleic Acid Res* and *Mol Biol* **56**:129–223). These alignments show that the N-terminus of all bacterial RecA proteins was never shorter by more than three amino acids than the *E. coli* RecA protein, which is consistent with our findings. The only exception to this rule was the *Bacteroides fragilis* RecA protein, which was reported to be 15 amino acids shorter than *E. coli* RecA protein, consequently losing four intermonomer contacts. We therefore re-examined the published nucleotide sequence of the *recA* gene of *B. fragilis* (Goodman and Woods, 1990, *Gene* **94**:77–82) and found an upstream sequence that was overlooked and that would encode, in the same frame, the absent portion of the N-terminus of the protein (Table 2). This presumptive part of the N-terminus (shown in small case) contains

Table 1. Different N-terminal truncations of RecA protein.

RecA phenotype			Aa position																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
RecA **WT**	+	M	A	I	D	E	N	**K**	Q	K	**A**	**L**	A	A	**A**	**L**	G	Q	**I**	E	K
RecA **Δ9**	−									M	**A**	**L**	A	A	**A**	**L**	G	Q	**I**	E	K
RecA **Δ11**	−											**M**	A	A	**A**	**L**	G	Q	**I**	E	K
RecA **Δ15**	−															**M**	G	Q	**I**	E	K

Shown in bold font are the amino acid residues located at positions 6, 9, 10, 13, 14 and 17 that are involved in the monomer–monomer interactions (Story *et al.*, 1992, *Nature* **355**: 318–325). The RecA$^{\pm}$ phenotype of truncated proteins was assigned based on the sensitivity of cells bearing the corresponding plasmids to UV light and to nitrofurantoin (Bakhlanova *et al.*, 1991, *Gene* **101**: 139–141) and on their ability to support growth of bacteriophage λ *int*$^-$*red*$^-$ (Davis *et al.*, 1991, *J Bacteriol* **173**: 5653–5662). Plasmids encoding the truncated RecA proteins were constructed using PCR. The forward oligonucleotide primers were 5′-AACATATGGCTATCGACGAAAACAAA-3′ for RecA WT, 5′-ATCATATGGCGCTGGCGGCCGCACTTGGCCAGATTGA-3′ for RecA Δ9, 5′-AACATATGGCGGCAGCACTTGGCCAGA-3′ for RecA Δ11, and 5′-TAACATATGGATCAGATTGAGAAACAATTTGGT-3′ for RecA Δ15. The reverse primer was 5′-GATAAGCTTCTGTCATGGCATATCCCTA-3′.

Table 2. Comparison of RecA proteins from *B. fragilis* and *E. coli*.

RecA aa position		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
RecA ***B. fragilis***		M	A	S	S	E	**K**	L	K	**A**	**L**	Q	A	**A**	**M**	D	K	**I**	E	K
RecA ***E. coli***	M	A	I	D	E	N	**K**	Q	K	**A**	**L**	A	A	**A**	**L**	G	Q	**I**	E	K

all the conserved amino acid residues required for monomer–monomer interactions. Therefore, we conclude that the initiation codon of the *recA* gene of *B. fragilis* was determined incorrectly and that all RecA proteins require amino acid residues starting at position 6 of the N-terminus of *E. coli* RecA protein for proper function.

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Widespread production of AS-48-like bacteriocins in strains of *Enterococcus faecalis?*

Sir,

The phenomenon of bacterial antagonism mediated by bacteriocins or bacteriocin-like substances has gained much attention in recent years in view of the potential practical applications of these agents in the control of undesirable microbiota. The most respresentative producers include members of the lactic acid bacteria (LAB) group, especially within the genera *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Carnobacterium*, *Lactobacillus* and also *Enterococcus*.

Among the different antimicrobial substances from enterococci described to date, the peptide AS-48 produced by *Enterococcus faecalis* S-48 (found in 1985 during a study on the potential production of bacteriocin-like substances) is remarkable for its cyclic nature and broad antimicrobial spectrum, as it inhibits not only Gram-positive bacteria, but also some Gram-negative species (Gálvez *et al.*, 1989, *Res Microbiol* **140**: 57–68). Indeed, AS-48 was initially considered as an antibiotic because of its broad spectrum. However, while antibiotics are secondary metabolites, AS-48 production occurs during the exponential phase of growth. For this reason, AS-48 should rather be considered as a bacteriocin. In fact, bacteriocins produced by Gram-positive bacteria differ greatly from the original definition of those from Gram-negatives, as they tend to be active against a wide range of Gram-positive bacteria and even on some Gram-negative species. Another interesting feature of AS-48 is its post-translational modification in which a very stable cyclic structure arises from a 'tail-to-head' linkage of the gene product, representing the first example of a cyclic molecule reassembly from ribosomal synthesis.

Moreover, and in agreement with its amino acid composition, bacteriocin AS-48 could also be considered as a cationic peptide with potent antimicrobial activity. Recently, several cationic peptides have been described in bacteria (i.e. nisin, lacticin 481, carnocin UI49, lactocin S, pep-5, subtilin, bacteriocin IYS2 and bacteriocin C3603), all of which show similarities in molecular size (3–6 kDa), isoelectric points (about 10) and biological activities. These inhibitory substances represent the conservation of a general mechanism of antibiosis during evolution.

The mechanism of action of cationic peptides typically involves channel or pore formation through the interaction of their positively charged residues and hydrophobic regions with the bacterial cell membranes, which are characterized by a large transmembrane potential and a high content of negatively charged lipids. Moreover, at concentrations around the minimum inhibitory concentration (MIC), these cationic peptides kill bacteria much more quickly than conventional antibiotics do. Therefore, this type of molecule could represent an interesting alternative to classical antibiotics.

In this active area of research, it is necessary to establish a rational scientific basis for the definition, classification and nomenclature of new substances in order to minimize possible discrepancies and confusion. For instance, new bacteriocins that have only minor conservative differences in their amino acid sequences, resulting in no significant change in their secondary structures, activity spectra and crossed immunity between producer strains, should be referred to as natural variants (in accordance with Jack *et al.*, 1995, *Microbiol Rev* **59**: 171–200). Also, these closely related bacteriocins should be given the same name, the first by which they were originally described.

In this respect, the recent publication in different journals of independent manuscripts dealing with three molecules almost identical to AS-48 produced by different enterococcal strains is surprising, because they all were given different names: enterococcin EFS2 (Maisnier-Patin *et al.*, 1996, *Int J Food Microbiol* **30**: 255–270), enterocin 4 (Joosten *et al.*, 1996, *Appl Environ Microbiol* **62**: 4230–4223) and bacteriocin 21 (Tomita *et al.*, 1997, *J Bacteriol* **179**: 7843–7855). Strain EFS2, isolated from the surface of a traditional cheese, produces a bacteriocin named