Crystal structure of the C24 protein from the Antarctic bacterium *Bizionia argentinensis* JUB59, a putative long tail fiber receptor-binding tip from a novel temperate bacteriophage

L. Pellizza[1], J.L. López[2], S. Vázquez[3], G. Sycz[1], B.G. Guimarães[4,5], J. Rinaldi[1], F.A. Goldbaum[1], M. Aran[1], W.P. Mac Cormack[3,6], S. Klinke[1]

[1]Fundación Instituto Leloir, IIBBA-CONICET, Buenos Aires, Argentina; [2]Instituto de Bacteriología y Virología Molecular IBAVIM, Universidad de Buenos Aires, Buenos Aires, Argentina; [3]Instituto NANOBIOTEC, Universidad de Buenos Aires, Buenos Aires, Argentina; [4]Synchrotron SOLEIL, Gif-sur-Yvette, France; [5]Instituto Carlos Chagas - Fund. Oswaldo Cruz, Curitiba, Brazil; [6]Instituto Antártico Argentino, Buenos Aires, Argentina

sklinke@leloir.org.ar

Tailed bacteriophages are one of the most widespread biological entities on Earth. Their singular structures, such as spikes or fibers are of special interest given their potential use in a wide range of biotechnological applications. In particular, the long fibers present at the termini of the T4 phage tail have been studied in detail and are important for host recognition and adsorption. Although significant progress has been made in elucidating structural mechanisms of model phages, the high-resolution structural description of the vast population of marine phages is still unexplored.

Our group studies the marine flavobacterium *Bizionia argentinensis* JUB59, a psychrotolerant Gram-negative microorganism isolated from surface seawater in Potter Cove, Antarctica, and whose genome has been sequenced. This bacterium constitutes a relevant source for the discovery of new proteins showing biological activity in extreme conditions of low temperature. In recent years, we have developed a medium-throughput structural genomics project to functionally classify *B. argentinensis* JUB59 proteins annotated with unknown function. We set up a screening protocol based on bioinformatics analysis, NMR and crystallography to identify and characterize suitable targets for structure determination [1-4]. In this context, and amongst other members, we selected a 277-residue protein named C24, whose sequence lacks homology to proteins of known function.

In the present work, we crystallized and solved the structure of C24 at 1.82 Å resolution by means of the single-wavelength anomalous diffraction method (manganese peak) with excellent statistics [5]. The protein folds as an 89-kDa homotrimer with a rocket shape. It bears a total length of 160 Å and a varying diameter along the particle axis, with a maximum value of 60 Å at its base. The structure of C24 closely resembles that of the receptor-binding tip from the bacteriophage T4 long tail fiber [6], although there are notorious differences in their domain organization, sequence, molecular dimension and number and type of bound structural divalent cations. We then confirmed the viral origin of C24 by bioinformatic and experimental approaches: (i) the C24 sequence is located inside a detected prophage by the ACLAME Prophinder tool, and (ii) the antibiotic mitomycin C induces the lytic cycle of a virus present in the bacterial genome, which was able to be isolated and visualized by transmission electron microscopy, revealing a morphology that is compatible with the order *Caudovirales* and, more importantly, these viral particles carry the nucleotide sequence of C24 in their genome.

As a general conclusion, the crystal structure of C24, together with induction and electron microscopy experiments, reveal that this protein may be the receptor-binding tip of a novel uncharacterized tailed bacteriophage present as a lysogen in *B. argentinensis* JUB59. These findings bring new avenues for the discovery of novel viral structures and provide valuable information to expand our current knowledge on the viral machinery prevalent in the oceans.

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