Cellular impact of A1, a nuclease of bacteriophage T5 essential for infection

Ombeline Rossier*†[1], Léo Zangelmi[1], Madalena Renouard[1], and Pascale Boulanger[2]

[1]Institute for Integrative Biology of the Cell (I2BC) – UMR9198, CEA, CNRS, Univ. ParisSud, Université ParisSaclay – 91198 GifsurYvette cedex, France

[2]Institute for Integrative Biology of the Cell (I2BC) – UMR9198, CEA, CNRS, Univ. ParisSud, Université ParisSaclay, – 91198 Gif-sur-Yvette cedex, France

Résumé

Phage T5 infects *Escherichia coli* and injects its genome in an original two-step mechanism: in the First Step Transfer (FST), only 8% of the phage DNA enters the host cell. The transfer pauses then for several minutes before DNA entry resumes to completion (Second Step Transfer, SST). The FST is accompanied by a very rapid and massive destruction of bacterial DNA (50 % decrease in labeled DNA within 4 min of infection). The identity of the phage T5 nuclease has remained elusive for sixty years, as none of the phage proteins encoded on the FST-DNA resemble known nucleases. However, *A1*, a gene carried by FST-DNA, appears to control host DNA degradation as well as the SST. The C-terminal half of A1 carries several motifs that are conserved in a large family of metallophosphatases including the DNA repair and recombination nucleases Mre11/SbcD/gp46. Purified A1 exhibits manganese-dependent DNase activity on linear or plasmid DNA *in vitro*. In this study we investigated the role of the 62-kDa protein A1 in DNA degradation in the bacterial cell. Upon ectopic expression of *A1* (cloned under the control of an arabinose-inducible promoter), there was a dramatic decrease in genomic DNA recovered from bacterial cultures. Using fluorescence microcopy of *E. coli* cells, we observed a rapid decrease in bacterial DNA staining with DAPI within 15 min of induction. Moreover, we frequently saw the formation of fluorescence foci, suggesting a major reorganization of the bacterial nucleoid. Mutations in putative catalytic amino-acid residues abolished nuclease activity in vitro as well as in vivo. Taken together, our results indicate that A1 is the long elusive early-encoded DNase of phage T5. Interestingly, T5 phage DNA is not modified and is sensitive to A1 *in vitro.* How the DNase activity of A1 is regulated to control the SST without digesting the T5 genome remains to be elucidated.

*Intervenant

†Auteur correspondant: ombeline.rossier@i2bc.paris-saclay.fr