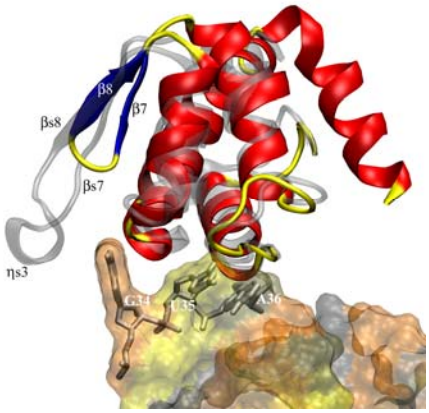
The Mimivirus protein involved in translation

The IGS laboratory structural group selected the most surprising genes of Mimivirus for further molecular characterisation of the corresponding proteins in order to verify their predicted enzymatic activity and to try to understand their origin. Our targets corresponded to genes identified for the first time in a viral genome such as the genes potentially involved in translation: 4 aminoacyl-tRNA synthetases, 3 genes potentially encoding initiation factors, an elongation factor and a termination factor as well as 2 enzymes involved in tRNA modification.

To date we verified the enzymatic activity and specificity of the Methionyl-tRNA synthetase, the Tyrosyl-tRNA synthetase (1,2). Our functional studies of the Mimivirus aminiacyl-tRNA synthetases confirmed the TyrRS specificity for tyrosine and its conformity with the archea/eukarya identity rules for $tRNA^{Tyr}$, while the Mimivirus MetRS is specific for methionine and as other eukaryotic types of MetRS, aminoacylates both eukaryotic and bacterial $tRNA^{Met}$. The Arginyl-tRNA synthetase and the cysteinyl-tRNA synthetase activity and specificity have also been recently measured and also confirm the bioinformatic predictions.

We also solved the structure of the Mimivirus TyrRS which presents a unique characteristic at its anticodon binding site, with a deletion of several residues in the recognition area of the 3rd base of the anticodon (based on bioinformatics studies). Accordingly, only weak effects were observed upon mutation of the G34 anticodon nucleotide on the yeast $tRNA^{Tyr}$. This specific feature might either be a simplification made possible by the absence of tRNA for TAG and TAA codons (STOP codons), or more boldly reminiscent of an ancestral two-letter genetic code.



C-term domain of the Mimivirus TyrRS (PDB 2J5B) **superimposed on the archeal TyrRS** (transparent grey, 2CYC). The tRNA is presented as molecular surface (transparent) and the anticodon inside the surface in grey.

The Mimivirus proteins involved in the viral DNA synthesis

We also characterized the Mimivirus nucleoside diphosphate kinase (NDK) (3,4). NDK are cellular enzymes necessary to maintain the nucleotide equilibrium in the cell. They are responsible for the synthesis of nucleoside triphosphates other than ATP. They also assume additional roles in metabolic regulation in bacteria, plants, fungi, vertebrates and invertebrates. A renewed interest for these enzymes arose ten years ago, as a result of their potential application in biomedical research due to their implication in growth and cellular differentiation in metazoan through their tumor metastasis suppressor activity. The question is now to understand the role of such an enzyme discovered for the first time in a virus. We first demonstrated that the Mimivirus NDK was fully functional, but while other NDK enzymes are non specific, the viral enzyme presents a peculiar affinity for desoxynucleoside pyrimidine triphosphate. We thus solved the viral enzyme 3D-structure in its apo and holo forms with dGDP and dTDP. Various other complexes are currently under refinement in order to elucidate at the atomic level the various determinants of the viral enzyme specificity. One possible role of this enzyme would be the production of dYTP for the replication of the high A+T viral DNA (70%) in a more conventional G+C content host (4).

Cartoon representation of the Mimivirus NDK oligomer (PDB 2B8P – 2B8Q)**.** Each monomer is represented with a different colour and the ligand is colored in grey in each of the six active sites.

To elucidate the molecular basis of the Mimivirus enzyme specificity toward deoxy-pyrimidine nucleotides, we generated a series of 7 mutant based on the structure comparison of the native enzyme with other nucleoside diphosphate kinase structures available in the PDB. Crystallization conditions were determined for the 7 mutants in complex with various nucleotides (deoxy- oxy- purine and pyrimidine) and the corresponding twenty complex structures have been determined and are now released into the PDB (5).

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