s1.m5.p5 **Applied homology modelling in the study of cell-to-cell movement of cucumoviruses.** Ákos Gellért,[ab] Katalin Salánki,[a] Emese Huppert,[a] Gábor Náray-Szabó[c] and Ervin Balázs[a], [a]*Agricultural Biotechnology Center, Hungary,* [b]*Department of Theoretical Chemistry, Eötvös Loránd University, Hungary, and* [c]*Protein Modeling Group, Hungarian Academy of Sciences, Eötvös Lóránd University, Hungary. E-mail: gellert@para.chem.elte.hu*

Keywords: Virology; Homology modelling; Cell-to-cell movement

Plant virus infections still cause considerable harm to the agriculture, therefore understanding the molecular mechanism of the virus spreading is of utmost importance. Most plant virus genomes contain a gene encoding the movement protein (MP), which plays a crucial role in the cell-to-cell spreading of viral RNA. According to subsequent assumptions in cucumoviruses the MP interacts with the coat protein (CP) and phloem protein (PP) allowing the MP to move the viral RNA from cell to cell.

The genome of cucumoviruses contains three single stranded RNA molecules. RNA 3 is composed of two genes, the MP gene and the CP gene. The main function of CP is to encapsulate the viral RNA forming a virus capsid. For the cell-to-cell movement of cucumoviruses both MP and CP are required, they are not exchangeable between *Cucumber mosaic virus* (CMV) and *Tomato aspermy virus* (TAV). The MP of CMV is able to function with the TAV CP (chimera RT), but TAV MP is unable to promote the cell-to-cell movement in the presence of CMV CP (chimera TR). To gain further insight into the non-infectious nature of the TR recombinant, RNA 3 chimeras were constructed with recombinant MPs and CPs. The chimeric MP and one of the CP recombinants were found to be infectious. The other recombinant CP enabled virus movement only after the introduction of two point mutations, Glu62Lys and Lys65Arg [1]. In order to predict the effect of the above mutations we generated computer models for TAV, CMV CP and three recombinant CPs, using subunit B of the Fny-CMV coat protein (PDB ID code 1F15) as the template structure [2]. The CP model structure of the movement deficient construct was compared with the other ones, but we could not detect any significant difference. In order to examine the surface properties of CP, we computed the electrostatic potential patterns for the models and observed pronounced differences in the patterns of infectious and non-infectious constructs. We designed a double mutant in order to modify the electrostatic potential pattern of the non-infectious CP. As *in vivo* experiments showed the mutated CP construct became infectious.

In addition, we constructed the P-TAV; Er-PSV; M, R and Trk7-CMV CP models, all belonging to genus cucumoviruses. After a PROSITE search we assigned sensible hits to the structure and analysed the environment of the hypothetical functional sites. A detailed examination indicated that most sites proved to be appropriate for CK II and PKC kinase binding as well as phosphorylation. Work for an experimental verification is in progress.

[1] K. Salánki, Á. Gellért, et al. *J Gen Virol* **85**, 1039-1048 (2004).
[2] T.J. Smith, et al. *J Virology* **74**, 7578-7586 (2000).

s1.m5.p6 **Structure of PACE12, an archaebacterial protein with GTPase activity and of unknown biological function.** S. Gras[1], P. Carpentier[1], B. Fernandez[2], J. Armengaud[2], P. Forterre[3], D. Housset[1], [1]*Institut de Biologie Structurale J.-P. Ebel, LCCP, CEA-CRNS-UJF, 41 rue Jules Horowitz, F-38027 Grenoble cedex 1,* [2]*Service de Biochimie et Toxicologie Nucléaire, CEA-ValRho Marcoule, BP17171, F-30207 Bagnols sur Ceze cedex.* [3]*Institut de Génétique et Microbiologie (IGM), Bat 409, Université Paris-Sud, Centre d'Orsay, F-91405 Orsay Cedex. E-mail: stephanie.gras@ibs.fr*

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An interesting subset of archaebacterial proteins conserved among eukaryotes has been identified by means of genome sequencing and comparative genomics: this protein family has been named PACE (Protein of Archaebacteria Conserved in Eukaryotes). These proteins that did not significantly evolve after more than 3 billions years are likely to catalyse a vital process such as transcription, methylation or DNA repair, conserved from archaebacteria until human.

Inspired by structural and functional genomics approaches, the goal of our project is to use x-ray crystallography to determine the function of these archaebacterial proteins, for which structural investigation is expected to be easier. Then, we hope to be able to extend this knowledge to their human homologs. Currently, 32 PACE proteins have identified, however their function is not known yet.

We have focused our effort on the PACE12 protein, a homodimer of 60kDa. In combining MAD and MIRAS methods, we have solved its crystal structure.

PACE12 adopts a Rossmann type of fold and contains a phosphate binding loop (P-loop) characteristic of ATP/GTPases activity. Indeed, a fold similarity search performed with DALI has confirmed the structural homology with ATPase/GTPase fold despite its low sequence homology. The GTPase activity has been confirmed by biochemical assays. Examining the structure of different PACE12 complexes with GTP and GDP should help us to unveil its biological function.