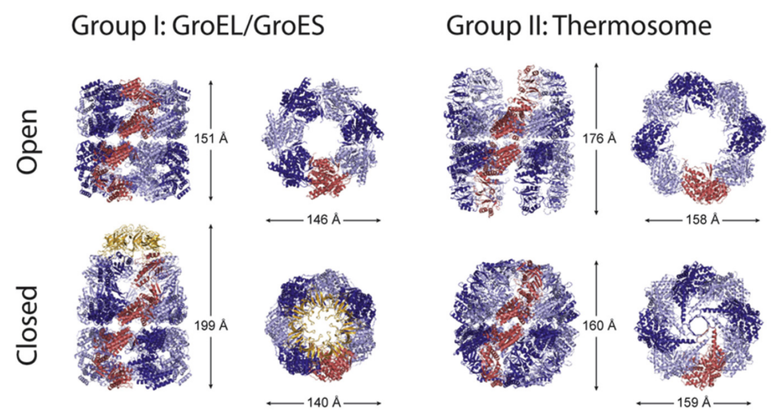
Cryo-EM Structure of the Single-Ring Chaperonin from Bacteriophage OBP *P. fluorescens*

T.B. Stanishneva-Konovalova[1] , P.I. Semenyuk[2] , Y. Pechnikova[3] , L.P. Kurochkina[4] and O.S. Sokolova[1]
[1.] M.V. Lomonosov Moscow State University, Faculty of Biology, Moscow, 119234, Russia.
[2.] M.V. Lomonosov Moscow State University, A.N. Belozersky Institute of Physical-Chemical Biology, Moscow, 119234, Russia.
[3.] Materials & Structural Analysis, Thermo Fisher Scientific, Eindhoven, Netherlands.
[4.] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, 117997, Russia

Prokaryotic and eukaryotic chaperonins

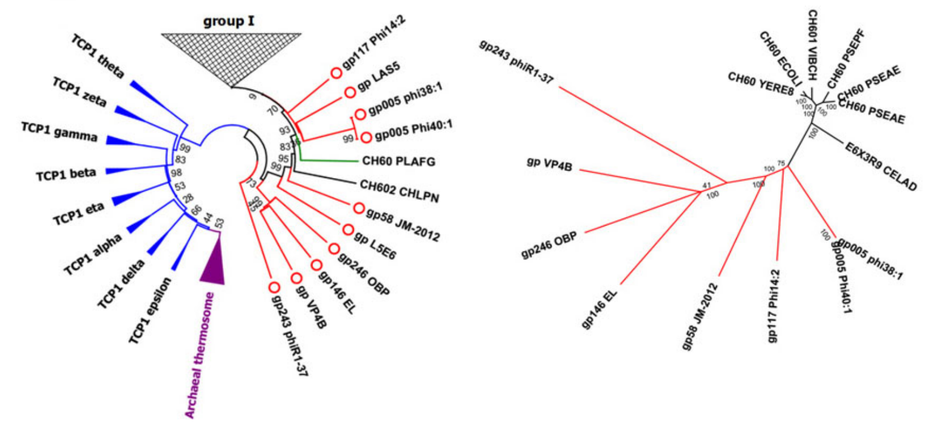
Chaperonins are the Hsp60 (heat-shock protein 60) family of molecular chaperones; they represent large multimeric complexes organized in characteristic barrel-like structures with an inner cavity where folding and assembly of certain denatured or newly synthesized proteins take place. All chaperonins can adopt an open substrate-receptive conformation, in which unfolded protein is recognized and encapsulated, and a closed conformation, in which the substrate is isolated from the intracellular environment. The transition between the open and closed states is induced by ATP binding and hydrolysis, which initiate a series of structural rearrangements.
Chaperonins are subdivided into group I (bacterial, mitochondrial and chloroplast chaperonins) and group II (archaeal and eukaryotic cytosolic variants). Group I chaperonins require a co-chaperonin, which caps the central cavity, group II chaperonins act without the assistance of a detachable cofactor, due to a built-in lid that closes the folding chamber.



Skjærven L. et al. Dynamics, flexibility, and allostery in molecular chaperonins // FEBS Lett. 2015. 589(19):2522-32.

Chaperonins of bacteriophages

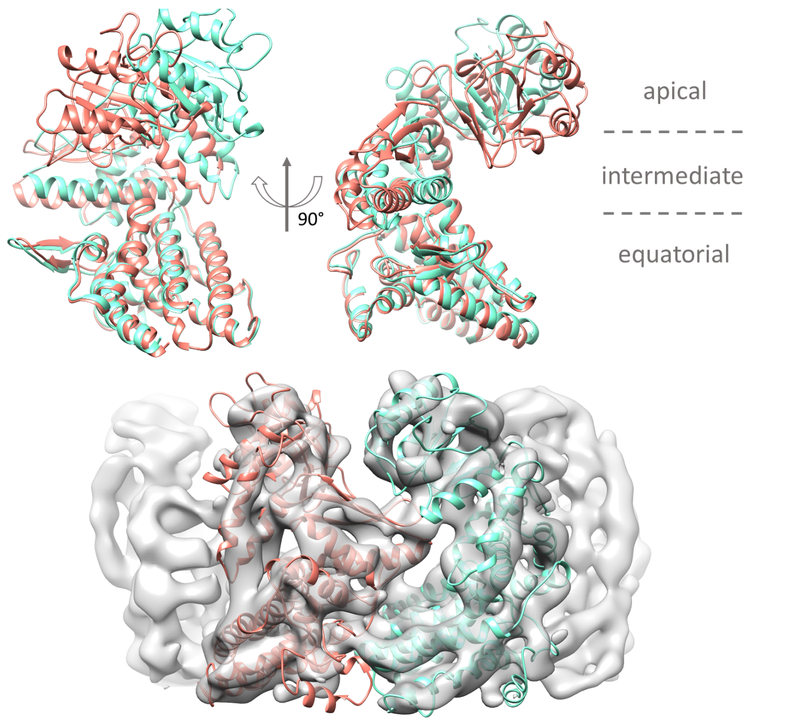
Putative GroEL-like chaperonin proteins have also been predicted in viruses of bacteria (bacteriophages) (Hertveldt et al., 2005; Cornelissen et al., 2012). Recently, two members of this group of proteins were obtained and characterized, and their chaperonin activity was demonstrated (Kurochkina et al., 2012; Semenyuk et al., 2016). Phylogenetic tree reconstruction using multiple alignment revealed that it is impossible to present the phage chaperonins as an individual subgroup due to their high diversity. However, chaperonins from phages EL, OBP and VP4B are relatively close to each other and can be combined into a monophyletic group.
The object of this study is the chaperonin of bacteriophage OBP Pseudomonas fluorescence, termed gp246 (gene product 246).

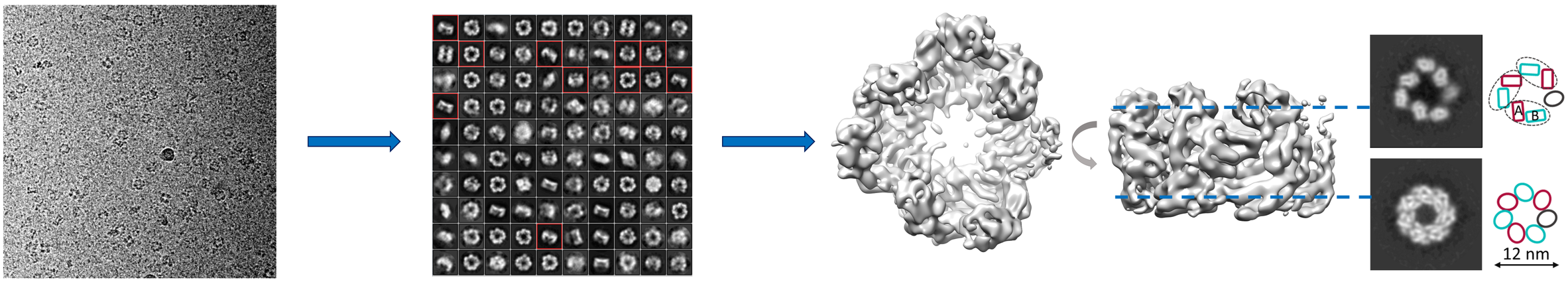


Left side: Phylogenetic tree of cellular and phage chaperonins. Phage chaperonins are red, group I chaperonins are black, group II chaperonins are blue (eukaryotic) and violet (archaeal), mitochondrial chaperonins are green. Right side: The phylogenetic tree of chaperonins from phages, their hosts and E. coli, based on the main multiple alignment. Phage chaperonins are red (Semenyuk PI, Orlov VN, Sokolova OS, Kurochkina LP. New GroEL-like chaperonin of bacteriophage OBP Pseudomonas fluorescens suppresses thermal protein aggregation in an ATP-dependent manner // Biochem. J. 2016. 473(15):2383-93.)

Cryo-EM structure of gp246

The structure of gp246 was studied by cryo-electron microscopy. Protein was isolated from E. coli lysate and purified using Q sepharose chromatography. Data collection was performed at NeCen (Leiden, Netherlands). Over the 1600 images were recorded on the Titan Krios microscope with the Falcon 2 direct detector using a magnification that resulted in a pixel size 0.784 A.
Over 44000 particles were processed in RELION-2.0 in order to get a symmetry-free 3D model at 7.3 Å resolution. The structure is a ring with a diameter of 12 nm, consisting of seven subunits. This distinguishes it from most chaperonins, usually consisting of two rings. The electron microscopy data are confirmed by analytical centrifugation results, according to which the chaperonin mass corresponds approximately to the mass of a heptamer.





Electron micrograph *2D classes* *3D model*

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