Pseudomonas aeruginosa phage EL chaperonin and a proposed model of its folding cycle.

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In the biosphere there are about 10 to 31st power of bacteriophages - bacterial viruses, and the number of bacteriophages in any liquid medium is usually several times higher than the total number of all bacteria in the same medium. Bacteriophages are considered harmless to the human body and can be chosen to serve as a tool for fighting dangerous bacteria, which is especially important now, when many bacteria have become resistant to antibiotics. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes infections in people with immunodeficiency. *P. aeruginosa* is the main respiratory pathogen in CF (cystic fibrosis). The phage EL, called ϕEL, described in this study infects *Pseudomonas aeruginosa*. We have discovered and currently study a first GroEL-ortholog in bacteriophages - chaperonin EL from *Pseudomonas aeruginosa* bacteriophage ϕEL, the product of its gene (gp) 146. In our opinion, this chaperonin EL is necessary for correct folding of anomalously long structural phage proteins, crucial for the phage survival. GroEL-orthologous chaperonin proteins were also predicted later in other bacteriophages and investigated by various methods. Three conformational states of EL-chaperonin (bound to ATP, bound to ADP, and apo form), which probably correspond to different stages of its ATPase cycle, have been investigated by cryo-EM.

It is known that chaperonins promote protein folding *in vivo* and *in vitro* and are widely found in bacteria, archaea and cytoplasm of eukaryotes. Chaperonins are large multimeric complexes organized into characteristic barrel-like structures with an internal cavity where the folding and

assembly of certain denatured or newly synthesized proteins occurs in an ATP-dependent manner. Chaperonins are divided into two families, group I and group II chaperonins. The GroEL / GroES system from Escherichia coli represents a well-studied group I shaperonin [1], [2]. Group II shaperonins[3] act without the aid of a removable cofactor due to the integrated cover that closes the folding chamber. Chaperonins of group I are present in bacteria, group II chaperonins function in archaea and eukaryotes.

We discovered[4] and studied[5],[6],[7] the first phage-encoded chaperonin from bacteriophage ϕEL infecting Pseudomonas aeruginosa, namely the product of this phage gene (gp) 146. The protein has a double ring morphology, which is typical for most known chaperonins, and functions as chaperonin without co-chaperonin in an ATP-dependent manner.

Proposed GroEL-orthologous proteins have also been predicted in other bacteriophages[6]. Multiple sequence alignment between ten predicted GroEL- orthologs encoded by phage genomes, group I chaperonins, and group II chaperonins has recently been processed to show the structural features of putative phage chaperonins. It has been found that the monomers of GroEL-like phage proteins have a common domain structure of known chaperonins. They also have three domains : equatorial, intermediate and apical (binding of the substrate). It was shown that the phage-encoded proteins contain highly conserved regions peculiar to both chaperonins from group I and group II. Thus, almost all of the residues involved in ATP binding in GroEL are conservative in putative phage chaperonins, whereas the substrate-binding site of phage proteins is less conserved. Amino acid residues corresponding to GroEL/GroES contacts are not conserved in phage chaperonins, suggesting that they function without co-chaperonins, such as group II chaperonins. However, it was found that all phage chaperonins do not have a spiral protrusion, characteristic of the members of group II. In turn, they have an insertion in the apical domain, like the group I chaperonins. According to multiple sequence alignment, the predicted phage chaperonins are probably closer to group I chaperonin than to group II chaperonins[6].

Normally, when a phage infects a cell, it will use the cell machinery to make new virus protein. This includes using the bacterial chaperonins. Some bacteriophages (λ, T4, RB49) use the host GroEL to fold their capsid proteins [8]. Unlike phage λ, which uses the GroEL/GroES bacterial system, phages T4 and RB49 encode their own co-chaperonins, the GroES-orthologs working in pairs with the host GroEL. We hypothesize[4] that ϕEL possesses a viral protein that is too large to be folded by the *Pseudomonas aeruginosa* chaperonin, so ϕEL must therefore carry its own chaperonin that can fold large viral proteins.

We carried out a structural study of EL-chaperonin using cryo-electron microscopy[7], and this investigation revealed various conformational states of EL-chaperonin depending on the nucleotide bound to the nucleotide binding site. The three conformational states under consideration (ATP, ADP and APO) were used as the basis for the hypothetical model of protein folding by chaperonin EL. ATP likely binds to both rings simultaneously and the binding of an improperly folded substrate acts as a trigger for ATP hydrolysis. We have no proof of two rings working at once but it makes sense. If only one ring folds the substrate then what is the other ring doing. Plus in order to form the single ring, ATP must be hydrolyzed to force the conformational change that creates the single ring. Nature is never wasteful. If the second ring is not folding a protein then ATP was hydrolyzed for nothing and was wasted. We therefore think that both rings must be actively folding a protein. It was further revealed that when ATP is hydrolyzed, the double ring structure of chaperonin EL dissociates into closed single rings. Conformational changes lead to an expansion of the inner ring chamber due to the movement of the equatorial domain, apparently, to be able to encapsulate and fold the viral proteins, which are too large for the GroEL-chaperonin of the host bacterium. Hydrolysis of ATP causes a rearrangement of the apical domain, which creates a lid that covers the inlet orifice in the chamber.

As to ring rejoining into a closed double annular conformation there can be several assumptions. Probably, the release of ADP from the nucleotide-binding pocket allows the rings to rejoin into a closed double annular conformation. Separated rings may find each other again by diffusion or perhaps never really completely separate *in vivo*, we just don't know. Binding of ATP to both rings opens the chamber to release the properly folded substrate and returns the chaperonin to the original open double ring conformation. Protein folding mechanism still store a lot of unknown stages, like ring separation into closed single rings, joining them back and many others, which were also noted in multiple studies in the description of the mechanism of group I folding with co-chaperonin GroES (see a comprehensive review[9], considering symmetric "football" intermediate [10] and human mitochondria[11] in particular).

The similarity of both members of group I and group II in combination with new characteristics makes it difficult to classify chaperonin EL as a member of group I or group II.

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