Protein uptake by bacteria
An endocytosis-like process in the planctomycete Gemmata obscuriglobus

John A. Fuerst* and Evgeny Sagulenko
School of Chemistry and Molecular Biosciences; The University of Queensland St. Lucia; St. Lucia, QLD Australia

Endocytosis is a fundamental process of membrane-trafficking in eukaryotes, but has not been known to occur in bacteria or archaea. The origin of endocytosis is central to the understanding of evolution of the first eukaryotes and their endomembrane systems. In a recent study we have established that an endocytosis-like process for uptake of proteins into cells occurs in a bacterium, Gemmata obscuriglobus, a member of the distinctive phylum Planctomycetes of peptidoglycan-less budding bacteria. Members of this phylum characteristically possess cells divided into compartments separated by internal membranes and in the case of G. obscuriglobus these compartments include one where a double membrane envelope surrounds its nucleoid DNA, as well as an outer ribosome-free region of cytoplasm. Proteins can be internalized by cells from the external milieu and collected into this ribosome-free region of cytoplasm. Proteins can be internalized by cells from the external milieu and collected into this ribosome-free region of cytoplasm. Proteins can be internalized by cells from the external milieu and collected into this ribosome-free region of cytoplasm. Proteins can be internalized by cells from the external milieu and collected into this ribosome-free region of cytoplasm.

Endocytosis is a cellular process for uptake of macromolecules from the external milieu by a mechanism involving plasma membrane infolding and vesicle formation. In the form of receptor-mediated endocytosis, it involves binding of the external macromolecular ligand to receptor molecules on the plasma membrane followed by trafficking of the receptor-ligand package to vesicles. The vesicles formed via plasma membrane infolding are called early endosomes; they are coated with a cage of clathrin and other proteins facilitating vesicle trafficking.1–3 Endocytosis seems to have been an ancient feature of eukaryotes, with evidence from phylogenetic analysis that its molecular machinery must have been present in the last eukaryote common ancestor (LECA).4–6 It is a process previously considered as eukaryote-specific, and was not known to occur in the domains Bacteria or Archaea, referred to together as prokaryotes. In bacterial cells import of amino acids and small peptides can occur but proteins have to be first degraded via extracellular proteases for them to be used as carbon, nitrogen and energy sources.

However, in a recent study we established that the planctomycete bacterium Gemmata obscuriglobus is able to uptake large folded proteins in a process somewhat similar to eukaryotic endocytosis.7 Our conclusions were based on the following findings: (a) Gemmata obscuriglobus cells can uptake full-length macromolecules in an energy-dependent manner. When cells were grown on agar medium and then incubated with proteins such as GFP, BSA, streptavidin, ovalbumin, horseradish peroxidase and immunoglobulin, they incorporated these proteins into the cell interior. This process is energy-dependent, since it is inhibited by sodium azide and non-permissive temperatures; ATP could restore the protein uptake after sodium azide inhibition.

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(b) This protein uptake ability appears to be receptor-mediated, and the receptor appears relatively non-specific with respect to different proteins. DNA in the form of fluorescent oligonucleotides and plasmid DNA was not taken up, and did not compete with proteins for uptake, suggesting that there are limits to the relatively non-specific nature of the receptor with respect to proteins. Competition between different proteins can be inferred from experiments showing that if *G. obscuriglobus* cells were incubated with two different fluorescent proteins at the same concentrations, equivalent signals are seen inside cells. If one protein was in considerable excess, the signal for the other protein could not be detected inside cells. Quantitative experiments show inhibition of uptake of fluorescent protein in the presence of a non-fluorescent different protein. These experiments established that the proteins use only one receptor for internalization; a model in which protein added in excess occupies the receptor to the exclusion of the competing protein is consistent with the experimental results and such a mechanism.

(c) Proteins taken up by *G. obscuriglobus* cells appear to localize to vesicles in a specific cell compartment in the outer region of the cell, the paryphoplasm. Immunogold labeling to localize GFP demonstrated this at EM level (Fig. 1A). This ribosome-free compartment is one known from previous ultrastructural studies to be characteristic for planctomycete cells; it is defined on its outer side by the cytoplasmic membrane and cell wall, and on its inner side by a single intracytoplasmic membrane. Since it is part of the cell cytoplasm as defined by the cytoplasmic membrane boundary, it is not to be confused with a periplasm. Using differential centrifugation and fractionation experiments we found that internalized proteins are associated with membranes and vesicles, but not with the soluble fraction. If proteins were to enter the cell via a pore-driven mechanism, they would be expected to remain in the soluble fraction rather than with membranes. Such pores would have to be large indeed if such proteins as GFP or immunoglobulin were to be allowed to pass through the membrane in a folded state. The association
of GFP with vesicles was also observed in the high-pressure frozen cryosubstituted cells (Fig. 1B), although the paryphoplasm in Gemmata cells is quite dense so this causes problems for visualizing membranes within the vesicles. Gold particles indicating GFP are clearly associated with vesicle membrane rather than the interior of vesicles, which is entirely consistent with a mechanism in which protein ligand associates with cytoplasmic membrane receptors, and remains attached to those receptors as the vesicle infolds. A membrane-trafficking vesicle-mediated mechanism is relevant to ways in which Gemmata cells must transport materials internally, since the cell cytoplasm is compartmented into membrane-bounded regions—e.g., transcription must occur in the nuclear compartment and protein synthesis is unlikely to occur in the ribosome-free paryphoplasm, so proteins for the cell membrane and wall must be transported across the intracytoplasmic membrane at least.

(d) We have found that an antibody raised against a G. obscuriglobus MC protein homolog8 reacts with vesicle-like structures within the paryphoplasm of G. obscuriglobus cells, and with vesicle membranes isolated by sub-cellular fractionation. Planctomycetes are known to be exceptional among Bacteria since they carry genes homologous to those coding for membrane coat (MC) proteins central to eukaryotic endocytosis.8 MC proteins are related to the clathrin and COP families, all members of which are associated with vesicle formation or membrane curvature, and share common secondary structural features of an α solenoid combined with a β-propeller,9 and some of which (e.g., clathrin) are necessary for receptor-mediated endocytosis.1

(c) Internalized proteins are degraded in the paryphoplasm—this implies the existence of lysosome-like compartments with proteases, and trafficking of internalized proteins from endocytic vesicles to those compartments. Conceivably such compartments contain other degradative enzymes—lysosomes possess aryl sulfatase among other degradative enzymes,10,11 and it is of interest that G. obscuriglobus has a gene annotated as an arylsulfatase, consistent with the occurrence in the genome of another planctomycete, Rhodopirellula, of many annotated sulfatases.12 This also suggests that some vesicles in the paryphoplasm should have acid pH internally, like lysosomes,10 and also LAMP and other lysosomal membrane proteins13,14 should be present; these predictions should be testable even though the small size of planctomycetes relative to eukaryotes makes this challenging.

From consideration of all these results, we proposed that Gemmata obscuriglobus has an ability to uptake proteins in a similar way to eukaryotic cells.7 We deduce here a model based explaining how a simple receptor-mediated endocytosis may occur in G. obscuriglobus (Fig. 1B). In the model, protein ligands bind to receptor molecules in the cytoplasmic membrane, and recruit clathrin-like membrane complex (MC) molecules which induce first a coated pit-like infolding of the cytoplasmic membrane followed by budding of a vesicle within the paryphoplasm. One of the resulting vesicles or vesicles derived later from these early vesicles may perform protein degradation.

We consider our finding as the first step in establishing the nature of endocytosis in bacteria. The next step will include the study of the molecular mechanism for this process. Although some data exist on occurrence of clathrin-like proteins in G. obscuriglobus,8 homologs of other proteins such as adaptins, SNAREs, Rab GTPases etc., known to be necessary for endocytosis in eukaryotes should be found in this species. This is conceivable since Rab GTPases may be present in other bacteria.20 Further experiments using pull-downs and investigation of existence of clathrin cages and triskelion formation from MC-like proteins should be undertaken. Another intriguing question is what kind of receptor for binding of proteins G. obscuriglobus possesses. This receptor is expected to be very unique as in contrast to the receptors of eukaryotes, it binds to a wide variety of proteins. Our work also raises a question of possible occurrence in these bacteria of an expected correlate of the endocytosis process, namely exocytosis where material is secreted from the cell via vesicle trafficking.

G. obscuriglobus is a member of the Planctomycetes, a phylum of budding and peptidoglycan-less bacteria sharing cell compartmentalization via internal membranes.21,22 G. obscuriglobus is distinguished by its possession of a membrane-bounded envelope around its nucleoid consisting of two apposed membranes which is thus analogous to the nuclear envelope of eukaryote cells.21,23 The planctomycete phylum forms a so-called PVC superphylum with other bacterial phyla Verrucomicrobia and Chlamydiae among several other phyla.24 Interestingly, verrucomicrobia also possess cells compartmentalized via internal membranes,25 and also possesses MC proteins. Clearly the endocytosis-like process we have found in one planctomycete species is relevant to our understanding of how eukaryote cell biology may have evolved. The steps to that understanding will involve deeper knowledge of mechanisms within the existing model organism G. obscuriglobus and also knowledge about the phyllogenetic distribution of such mechanisms within related bacteria.

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