

In vitro observation of the bacterial flagellar type III protein export

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The bacterial flagellation is a well-organized process. The bacterial flagellum is a super molecular complex composed of about 30 different proteins with tens thousands of protein subunits. Since the flagellum extends from the cell membrane to the exterior, most of the external component proteins, such as flagellar axial proteins, have to be exported to the distal end for self-assembly through the flagellar type III protein export apparatus. The export apparatus transports 20 to 30 thousands of protein subunits of more than 12 different types of proteins. Thus the protein export control is essential to construct the flagellum. To investigate the molecular mechanism of the flagellar type III export and the flagellar formation, we constructed an *in vitro* protein transport assay system that enables the measurement of protein transport under well-controlled conditions by using inverted membrane vesicle (IMV).

Using this system, we have observed the flagellar protein transport and the flagellar axial structure construction in IMV. The hook formation and the hook length control have successfully been reproduced in IMV by simply adding the component proteins into the IMV solution. We also found that the secretion order of the class-II proteins (the rod-hook type proteins) related to the proximal rod formation. Moreover we have detected the protein translocation into the IMV by fluorescence. This technique will enable the accurate and quantitative analysis of the flagellar type III protein export.