Investigating the molecular interactions of the ϕ29 DNA packaging motor

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Introduction

The bacteriophage ϕ29 of *Bacillus subtilis* packages its genomic dsDNA in an ATP-dependent fashion, with components of the virus forming a powerful molecular motor. The viral procapsid is composed of the capsid proteins with an associated connector (portal). Packaging of the viral genomic dsDNA (covalently complexed with gp3) involves the presence of RNA molecules (pRNA) and hydrolysis of ATP by an associated ATPase (gp16). The predicted secondary structure of pRNA is a three-helix junction with complementary bases in two of the loops. The possibility that base-pairing between adjacent pRNA molecules is involved in the tertiary structure of pRNA has been investigated through the mutation of these loop sequences and as a consequence, there have been many reports in the literature on the multimeric nature of pRNA in DNA packaging. The issue of pRNA stoichiometry is a matter of debate and it has been postulated that both pRNA hexamers and pentamers could play a role in DNA packaging. We have taken a novel approach in order to provide information essential for the rational elucidation of the mechanism of DNA packaging, based on determination of the affinity between components and the use of single molecule photobleaching experiments (SMPB).

Towards a model for motor function

The presence of solution state multimers of pRNA, formed by multimerization in the absence of other ϕ29 components, has been analysed by light scattering experiments. Further analysis of pRNA multimerization has been achieved using analytical ultracentrifugation (AUC), leading to data for the affinity of the solution state pRNA : pRNA interaction under a range of magnesium ion concentrations. The binding affinity of pRNA to connector gp10 has been investigated by two independent techniques (see previous Astbury reports).

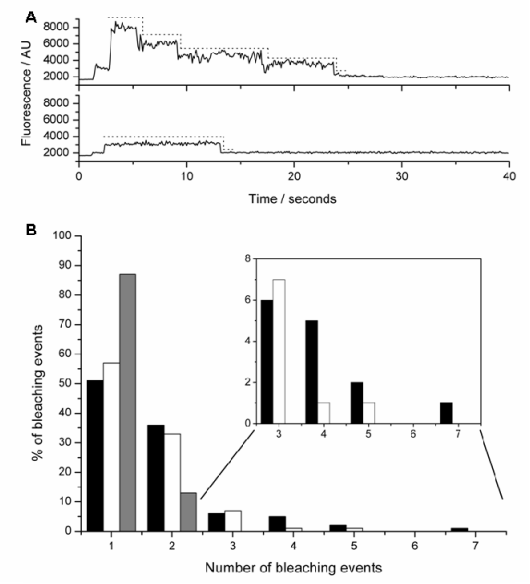

Figure 1 Example traces showing fluorophore photobleaching for labelled multimerisation-competent pRNA with 10mM Mg^{2+} + C-His gp10 connector (A, upper) and mutant pRNA + 10 mM Mg^{2+} (A, lower). The dashed line provides an indication of the steps identified in this particular trace and used in data analysis. Data from SMPB experiments were analysed by determining the number of photobleaching events observed in different experimental assemblies (B). In the case of the mutant pRNA + 10 mM Mg^{2+} (grey), approximately 90% of the photobleaching events represent monomeric species. When the number of photobleaching events for the multimerisation-competent pRNA + 10 mM Mg^{2+} is analyzed (white), there is an increase in the proportion of dimeric species observed and a significant number of pRNA trimers are also present. A stabilization of higher order pRNA species is observed as an increase in the occurrence of four or more photobleaching events (inset show enlarged view of higher order region of histogram) when this RNA + 10 mM Mg^{2+} is incubated with gp10 connector (black).

SMPB experiments, in which the photobleaching of individual fluorophore-labelled pRNA molecules is monitored, have been used to further confirm the ability of pRNA to form higher order assemblies and also probe the effect of the presence of connector on pRNA

multimerization. The role of intermolecular base-pairing of pRNA in the formation of higher order multimeric forms has been probed in all experiments by the comparison of wild type and multimerization-incompetent mutant pRNAs.

Our current studies are focusing on conformational changes in both the connector and pRNA. We believe that unlikely that there is a rotation between the connector and pRNA during DNA packaging and we propose that magnesium-induced conformational changes in both connector and pRNA could drive the packaging event.

Collaborators
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Publications
Robinson M.A., Wood J.P.A, Capaldi S.A., Baron A.J. Gell, C. Smith, D.A. and Stonehouse, N.J. (2006) Affinity of molecular interactions in the bacteriophage ϕ29 packaging motor. *Nucleic Acids Res*. **34**; 2698-2709.