Packing Pattern of DNA in Bacteriophage T2

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Osmotic shock was applied to phage T2 in such a manner that the compact-mass of DNA was released from phage. The shape of this compact-mass of DNA was studied under electron microscope. It appeared that the DNA was packed into an elongated icosahedron similar to the phage head.

Bendet *et al.* [1] had postulated that the DNA within the bacteriophage head might be aligned parallel to the long axis of the phage. North and Rich [2] also suggested that some part of the DNA might remain oriented within the head along the length of the phage. This was also corroborated by the studies of Maestre and Kilkson [3]. Further studies indicated that 10 to 30% of the total DNA-content of a phage could remain along the long axis of the phage [4, 5]. Klimenko *et al.* [6] ruptured T2 phage head with phosphotungstic acid and observed ring-like pattern of DNA. Richards *et al.* [7] disrupted phage head by mechanical shock and observed a set of closely-packed concentric circles of DNA-strands. It was postulated that DNA within the phage head might be wound like a ball or a spool. Thus it appeared that DNA within the phage head remained oriented in a regular manner. In the present work, a new technique was developed which disrupted phage coat gently and enabled to visualize the overall packaging-pattern of the ejected DNA under the electron microscope.

A mixture of 10^{10}/ml T2 phage and 5 M ammonium acetate was equilibrated at room temperature for half an hour. One drop of this mixture was placed on a carbon-coated 400-mesh grid. After about 5 min excess phage-salt mixture was blotted out. The grid was then dipped into double-distilled water taken in petri dish for 10 s. In this step osmotic shock ruptured the protein coat of the phage head and consequently compact mass of DNA was released. The excess water was blotted with a filter paper. The sample was stained with 1% uranyl acetate and rotary shadowed with Pt-Pd. The various steps of the method have been illustrated diagramatically in Fig. 1. In this method osmotic shock was employed to phage which had already been adsorbed to carbon support film on grid and was called "osmotic shock-on-grid" method. Samples were examined in Siemens Elmiskop 101.

Under the electron microscope it was observed that 90% of the total phage present were disrupted due to osmotic shock. The DNA was released from the phage head and was observed as a compact-mass lying close to the disrupted phage head. Fig. 2 is a representative electron micrograph of T2 phage obtained by osmotic shock-on-grid studies. The disrupted phage is seen with negative contrast while the DNA-mass is seen with positive contrast. The most interesting observation was that the outline of the compact-mass of DNA had some angular turning points. Due to these angular turning points the DNA-mass resembled the phage capsid. Such peripherial angular turns were resolved clearly in 50% of the disrupted phage.

Thus it appeared that the DNA remained in a compact form within the phage head and the shape of this compact-mass of DNA was like an elongated icosahedron. In other words the DNA is packed like an elongated icosahedron similar to the shape of the head. This is consistent with the findings of Laemmli and Favre [8] who inferred that DNA is

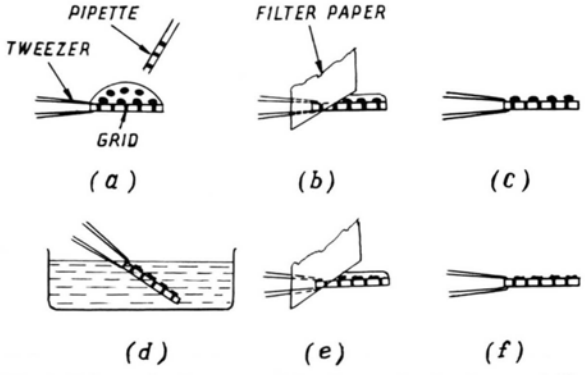

Fig. 1. Schematic diagram of the "osmotic shock-on-grid" method for visualizing the arrangement of DNA. (a) A drop of phage-salt mixture is put on the grid, (b) Excess phage-salt mixture is blotted out, (c) After blotting the excess phase-salt mixture, (d) The grid with adsorbed phage is dipped in water. Osmotic shock ruptures the phage head and compact-mass of DNA is ejected, (e) Excess water is blotted out, (f) Sample is stained with uranyl acetate and shadowed with Pt-Pd.

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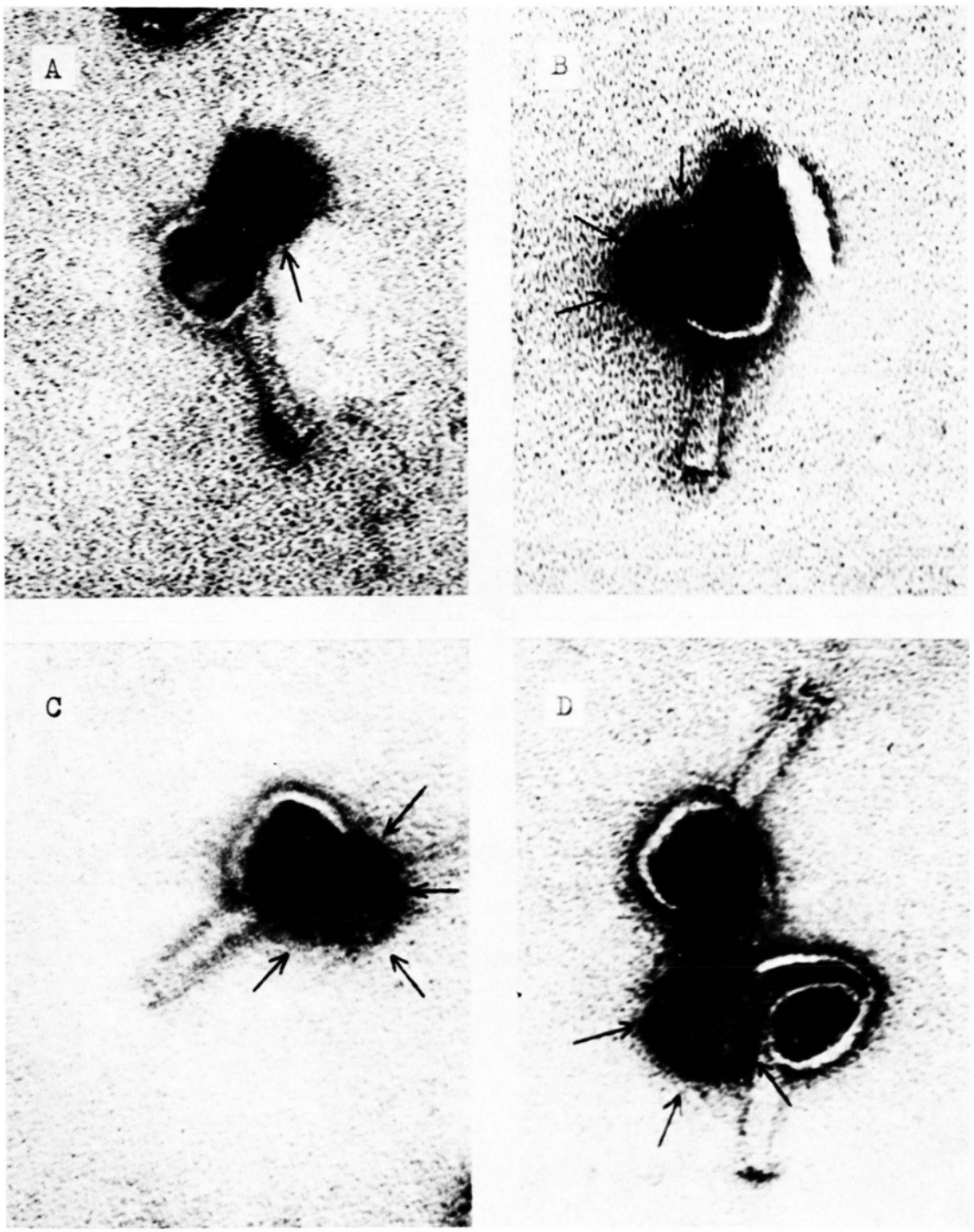

Fig. 2. Electron micrographs of the ejected DNA from bacteriophage T2 prepared by "osmotic shock-on-grid" method. The phage particles are seen with negative contrast while the DNA-mass is seen with positive contrast. The DNA-mass is seen to have some angular turns. The angular turning points are indicated by arrows. In (C) and (D), the portion of the DNA-mass marked by arrows resembles the phage capsid. Magnification: 1,90,000 X.

packed into a preformed head. The icosahedral shape of the DNA-mass also hinted at the fact that DNA is packed from outside to inside within the head.

Some of the ejected DNA-mass showed fewer angular turning points. This may be due to the fact that some portion of the DNA-mass was within the disrupted phage head and was obscured by stain.

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