MEMBRANE ASSOCIATION OF CIRCULAR FORMS OF ColE$_1$ DNA: ELECTRON MICROSCOPIC EXAMINATION

Yu.F. DRYGIN, S.L. BOGDANOVA and A.A. BOGDANOV
Laboratory of Bioorganic Chemistry of the Moscow State University, Moscow, USSR

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1. Introduction

Current concepts of the replication of the bacterial chromosome suggest the attachment of the point of origin of DNA replication to the cell membrane [1, 2]. Recently Smith and Hanawalt [3] developed a method for the isolation of DNA–membrane complexes, based on the separation of DNA–membrane structures from the bulk of the DNA by a short sucrose-gradient centrifugation of gently lysed cell preparations. However, the electron microscopy of the membrane-bound DNA fractions indicated that they were contaminated with free high-molecular weight DNA which sediment together with complex (Drygin, unpublished data).

This communication described the electron microscopic examination of ColE$_1$ DNA–membrane complexes. The synthesis of the DNA that determines the production of colicin E$_1$ can be induced in collicinogenic strains of *E. coli* by various physical and chemical treatments [4]. ColE$_1$ DNA has a circular form and low molecular weight (4.2×10^6). Therefore membrane-bound ColE$_1$ DNA can easily be separated from free ColE$_1$ DNA and distinguished from host chromosomal DNA.

2. Isolation of DNA–membrane fractions

E. coli K12SE$_1$ was cultured and ColE$_1$ DNA synthesis induced with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidinium (NG) (30–50 μg NG was added to 1 ml of culture containing $1.5–2 \times 10^8$ cells/ml) as described previously [5]. 0.6 μCi ^{14}C-thymidine (44 mCi/mmole) and 7.5 mg deoxyadenosine were added to 10 ml of the suspension simultaneously with NG. After 30 min the cells were harvested and washed

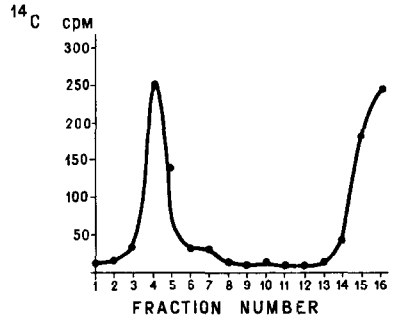

Fig. 1. Sucrose gradient analysis of gently-lysed cells of *E. coli* K12SE$_1$.

with 30 ml of cold ^{12}C-thymidine solution in 0.05 M tris, pH 8.1. Lysozyme (0.3 ml of a 0.16 mg/ml solution in 5% sucrose and 0.05 M tris, pH 8.1) was added and the suspension frozen and thawed three times. Clear lysate (0.1 ml) was layered onto a 5–30% sucrose gradient containing a 1 ml underlayer of 60% sucrose. Sucrose solutions were made up in 0.05 M tris, pH 7.0, containing 0.005 M EDTA and 0.15 M. Centrifugation was performed in a Hitachi model 55P ultracentrifuge in a type RPS-40 rotor at 33,000 rpm and 4° for 1 hr. At the end of the run 0.3 ml fractions were collected and frozen. The samples were counted with a dioxane scintillation solution in a Nuclear Chicago Mark II liquid-scintillation counter (fig. 1). For electron microscopy 0.1 ml aliquots were collected from each sucrose-gradient fraction and mixed with 0.1 ml of 0.1 M ammonium acetate, pH 7.0 and 0.02 ml of 1% diisopropylphosphoryl trypsin in 1.0 M ammonium acetate, pH 7.0. 0.1 ml of this mixture were spread onto the surface of a 0.12 M ammonium acetate solution [6]. Samples were picked up on collo-

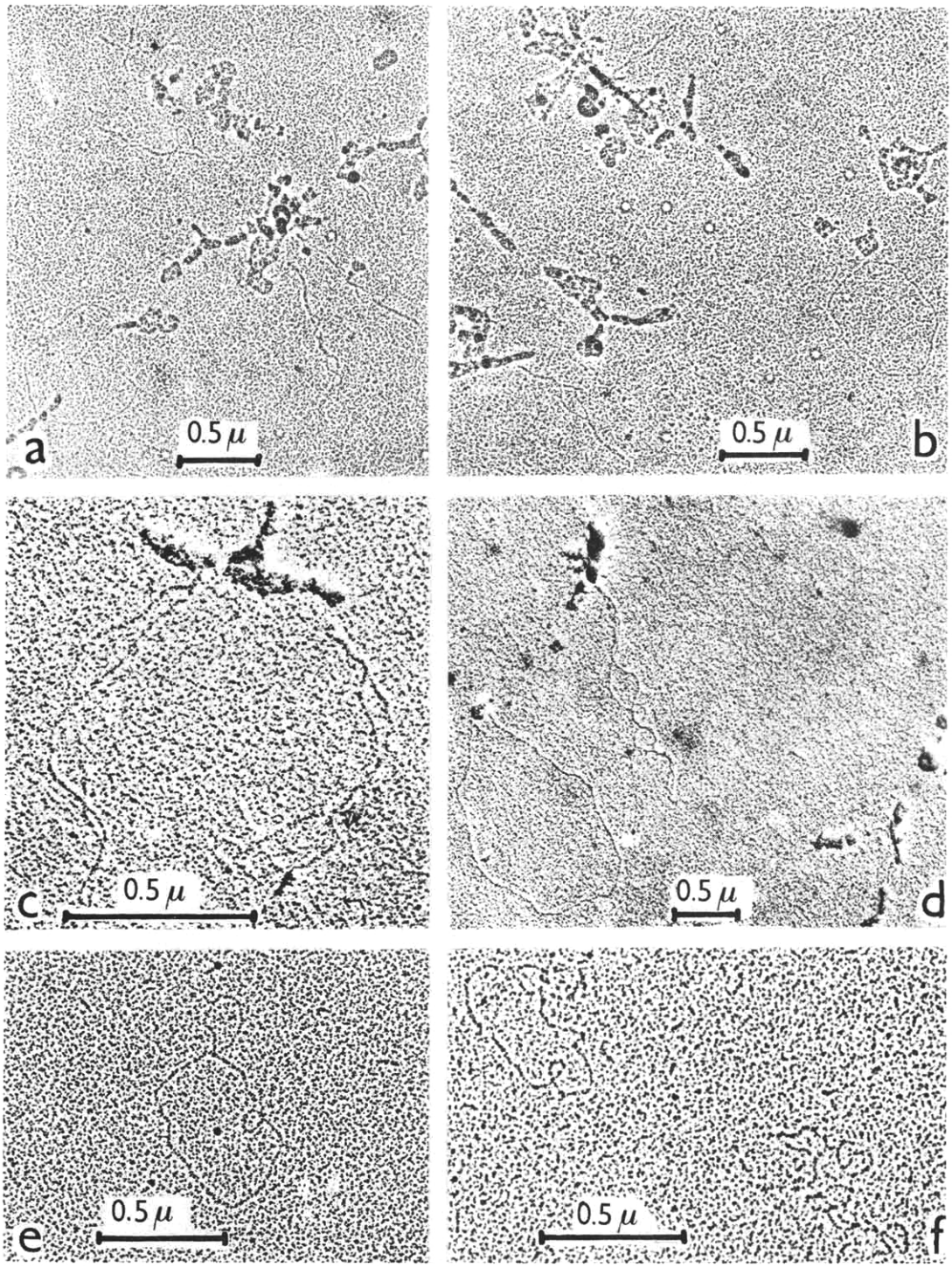

Fig. 2. Electron micrographs of supercoiled and open plasmid DNA: (a, b, c, d) fast -sedimenting fractions of the sucrose gradient (fraction nos. 3–6); (e, f) free plasmid DNA (fraction no. 14–16).

dion-carbon-coated copper grids, washed in methyl alcohol, dried and shadowed with palladium using a rotatory shadower. Samples were examined in a Hitachi HS-8 electron microscope at 50 kV and micrographs were taken at optimum magnifications.

3. Results and discussion

As fig. 1. shows, the rapidly-sedimenting membrane fractions (nos. 3–6) are enriched with ^{14}C-thymidine-labelled-DNA. When samples of the material in these fractions were examined by electron microscopy (fig. 2) membrane-bound circular DNA molecules of size characteristic of ColE$_1$DNA were observed side by side with high-molecular-weight host chromosomal DNA. A siginificant proportion of the membrane-associated ColE$_1$DNA molecules were in the form of double-stranded open circles and some of them were found in the covalently closed supercoiled form. Both closed and opened forms of circular DNA were also found in the light fractions but they were in the free state. Samples from the middle part of the gradient contained insignificant amounts of DNA-like material.

Simultaneous electron microscopic examination of the sucrose gradient from the control lysate (*E. coli* K12SE$_1$ cultured in the same conditions but without NG) showed negligible amounts of colicinogenic DNA in the same fractions.

4. Conclusions

Two main conclusions can be drawn from the results presented: (1) the general concept of the membrane attachment of the replication origin of bacterial and phage chromosome DNA should be extended to extrachromosomal plasmid DNA; (2) ColE$_1$DNA–membrane complexes are stable during the preparation of samples for electron microscopy; these complexes therefore are very suitable for ultrastructural studies of the association of DNA with cell membrane components.

References

[1] N. Sueoka and W.G. Quinn, Cold Spring Harbor Symp. Quant. Biol. 33 (1968) 695.
[2] R. Knippers and R.L. Sinsheimer, J. Mol. Biol. 34 (1968) 17.
[3] D.W. Smith and P.C. Hanawalt, Biochim. Biophys. Acta 149 (1967) 519.
[4] M. Nomura, Ann. Rev. Microbiol. 21 (1967) 257.
[5] S.L. Bogdanova, I.V. Fetisova and V.U. Gavrilov, Mikrobiologiya, 39 (1970) 622.
[6] A. Kleinschmidt and R.K. Zahn, Z. Naturforsch. 14b (1959) 770.