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Preparation of E. Coli Minicells Containing RNA Polymerase Genes

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Introduction

An F^- strain of E. coli K-12 which produces DNA deficient minicells by an improper cell division has been used in developing methods to isolate plasmid DNA.[1,2] By using sucrose gradient sedimentation, minicells can be easily separated from the parental cells that produce them. This E. coli mutant has a defect which is unable to synchronize DNA replication and cell division. Cell division occurs before DNA synthesis is finished, and consequenty one of the daughter cells does not receive any DNA. These small and DNA-less cells are called minicells.[1] They are not viable cells, but they do maintain their biological activities for approximately 48 hrs. It is possible to prepare plasmids which contain any region of the bacterial chromosome, and these can be easily placed in minicells.[3]

Since minicells can be easily separated from large cells, this system offers a great advantage for the isolation of a pure, genetically homogeneous region of the bacterial chromosome. In this work, a method for preparation of the minicells, carrying RNA polymerase genes is proposed.

Materials and Methods

The minicells producing a strain of E. coli K-12 (P_{67854}, F^-, thr^-, leu^-, Mal^+ Rif^r) was used as recipient bacteria, and several markers were introduced by transduction and conjugation.

E. coli $CGSC_{4285}$ which has episom F 111, was used as donor. Episome F 111 carries RNA polymerase gene(s), which is rifampicine sensitive, and confer to the host cell rifampicin sensitivity.

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Isolation of minicells

An overnight culture of E. coli K-12 (at starvation phase) was spun for 5 min. at 1500 rpm to obtain cnude minicells fraction. The supernatant was then spun down at 10,000 rpm 15 min. to pellet the minicells. Minicells then were resuspended in a phosphate buffer and layered onto a 30 ml of 5-20 %linear sucrose gradient, and spun at 5,000 rpm for 15 min. at 4°C. After centrifugation, fractions were collected and assayed for TCA insoluble radioactivity, or the minicells band was removed and pelleted, the minicells fraction was re-suspended in the appropiate growth medium at an absorbancy of 0.2 at 620 nm and assayed for biological activities and the contaminating cells.

Incorporation of radioactive precursors

F^- and F^+ strains of E. coli K-12 were grown in minimal medium containing 5μC/ml ^{3}H-TdR and 0.1μC/ml ^{14}C-leu and minicells were isolated and tested for radioactivity. All the radioactive samples were placed on Whatman 3MM filter discs and precipitated with a 10% cold TCA. The filters were washed twice with a 5% cold TCA, and twice with 100% ethonal then dried and placed into vials. Approximately 3ml of toluen,phosphorus was then added to the vials and the samples were counted.

Bacterial conjugations

Overnight cultures of donor and recipient bacteria were diluted to O.D. 100 Klet units. Then they were mixed and shaked gently for an hour on the rotatory shaker, And, the conjugation mixture was plated on the selecetive media which is permissive only for recombinants.

Results

Minicells can be easily separated from large cells, by differential centrifugation and the sucrose gradient sedimentation as described in materials and methods (Figure 1).

There are some differences in the ability of plasmid containing minicells to synthesize macromolecules, depending on the method of isolation. As indicated (materials and methods) the, minicells are routinely isolated by a series of sucrose gradient sedimentation, in linear 5-20% gradients. This method has an obvious disadvantage of exposing the minicells to an osmotic change resulting plasmolysis. The reduced

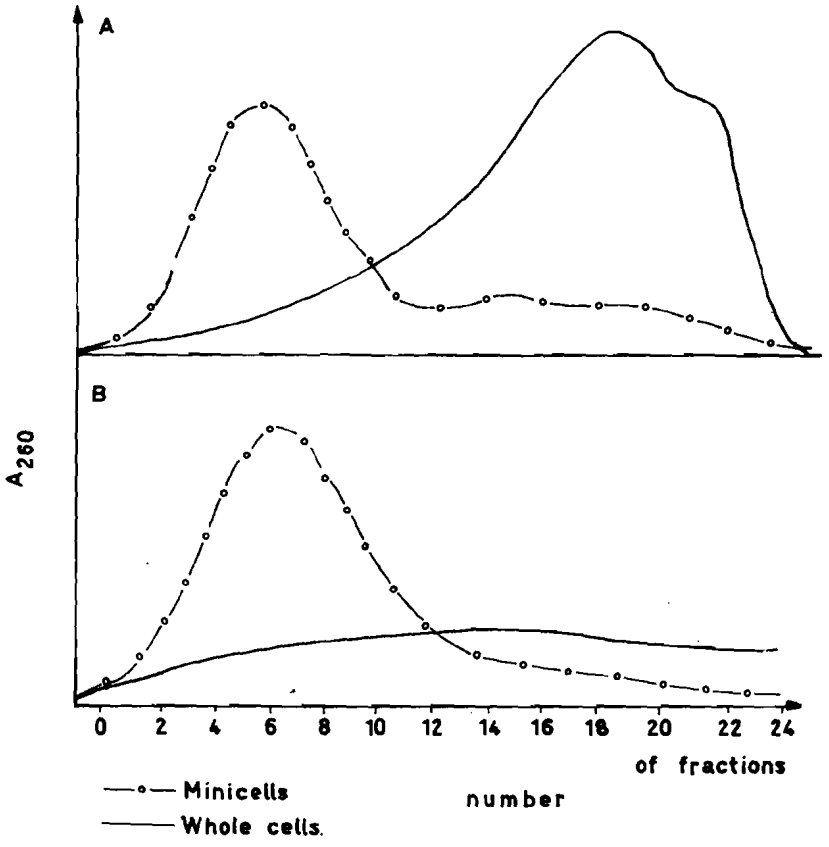


Figure 1

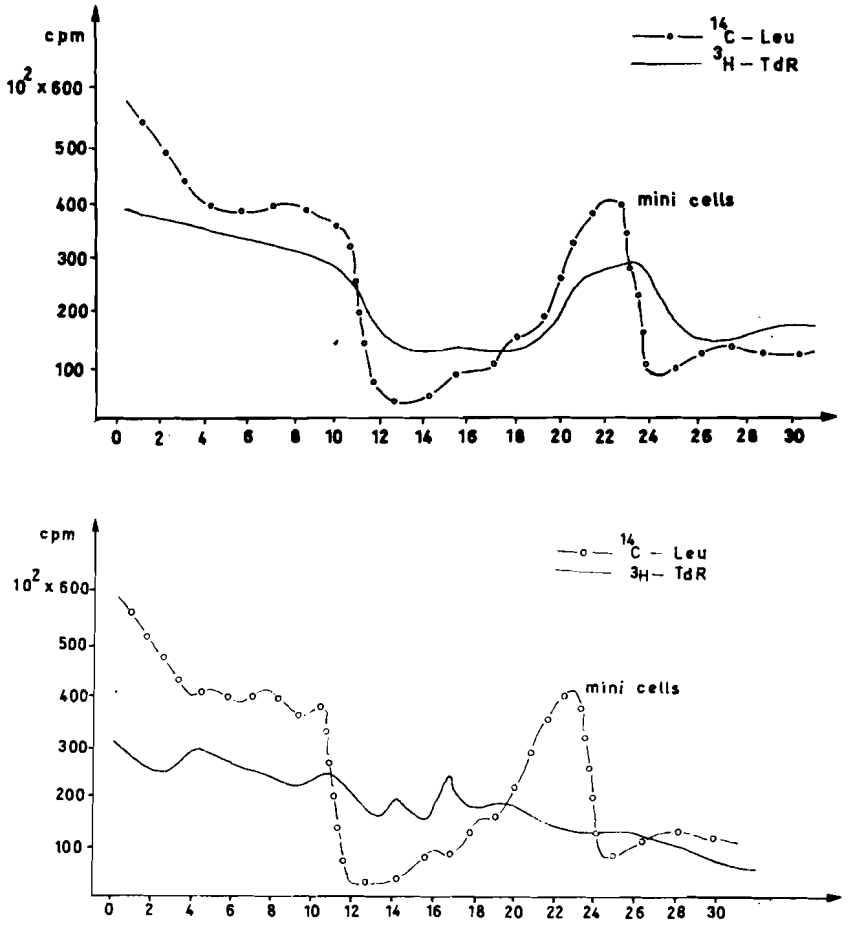
A_{260} Profiles for sucrose gradient of minicells producing strain of E. coli. a) First sucrose gradient, b) Second sucrose gradient

synthetic capacities of plasmolyzed cells of E. coli have been reported by Rubenstein et al.[4] Therefore ficol gradient has been tried for isolation of minicells. Another method of the isolation of minicells has been suggested by Levy.[5] Bacteriocidal agents, like penicillin that acts only on dividing cells is used to lyse cells in culture containing normal cells and minicells. On the other hand, protein synthesis in minicells which are treated with this antibiotic is reduced by about 60 %. The use of such treatment, therefore, may not be useful.

Preparation of E. coli minicells carrying RNA polymerase genes

RNA polymerase genes can be placed into the minicells with the help of a plasmid or a sex factor which contains the structurel genes for

RNA polymerase. A trick which makes the use of the Rifampicin sensitivity for detecting the presence of RNA polymerase genes in a cell was used. Rifamycin and its semi - synthetic derivatives like Rifampicin inhibits the RNA polymerase of E. coli. They act on the RNA polymerase itself instead of interacting with DNA.[6] Rifampicin prevents the formation of the initiation complex on the DNA- directed RNA polymerase reaction. It is possible to isolate RNA polymerase mutants by selecting for Rif. resistant mutants.



Figures 2 a, b

Radioactivity profiles for sucrose gradients of minicells produclng strains a) with and b) without plasmids. A culture of E. coli K-12 was growing overnight at 37°C on a rolatory shaker in the precense of 5μC/ml ^{3}P-TdR and 0.1μC/ml of ^{14}C-leu.

There are three isogenic merodiploids which differs only in the state of their Rif. genes.[7]

Genotypes	Phenotypes
Rif^r/Rif^r	Resistant
Rif^s/Rif^s	Sensitive
Rif/Rif	Sensitive

Heterodiploids are always phenotypically Rif. sensitive, even though they have resistant genes. Then episome F 111, which has Rifampicin sensitive RNA polymerase genes on it, has been transferred into Rif. resistant F^- minicell-producing cells, and phenotypically Rif. sensitive ones were seleceted Since recipient bacteria were Rif. resistant, Rifampicin sensitivity was taken as evidence of the transfer of episome F 111 to these cells. Five percent of the conjugation products were Rif. sensitive.

Radioactive experiments showed that, minicells, isolated from this stage had episomal DNA in them (Figures 2 a, b).

Discussion

The minicell is a potentially useful system for studying a variety of cellular processes. The ability of these minicells to produce viable bacteriophages after infection, is taken as indication that their synthetic activities are of biologîcal interest. Minicells have the same cell wall and membrane properties as their parental cells. Therefore, several bacteriophages can infect the minicells as well as parental cell. Roozen, Fenwich and Curtis have shown that minicells are capable of producing viable T_4 phages.[3]

A variety of control problems has been studied by using lambda phage. It is known, that lambda phage has several control genes and proteins, but they have not yet been separated from other host proteins and chracterized.[8]

Lambda phage can also infect the minicells, but since it must be supported by host RNA polymerase, it will not grow in the F^-minicells.

However, it is possible to put RNA polymerase genes into minicells, and then infect them with phage. Since minicells do not have their own DNA (even plasmid containing ones have a very small portion of DNA), it should be easier to isolate lambda phage gene products from minicells, rather than from normal host cells.

In conclusion then, plasmid containing minicells are capable of synthesizing DNA, RNA and protein will therefore provide a valuable system for studying replication, transcription or translation problems.

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REFERENCES

1. Adler, H. I., Fischer, W. D., Cohen. A.. et al.: Miniature Escherichia coli cells deficient in DNA. *Proc. Natl. Acad. Scl. U.S.A.* 57 (1967), 321-326.
2. Inselburg, J.: Segregation into and replication of plasmid deoxyribonucleic into acid in chromosomeless segregants of Escherichia coli, *J. Bacteriol.* 102 (1970), 642-647.
3. Roozen, K. J., Fenwick. R. G., Jr., and Curtiss, R., 3d.: Synthesis of ribonucleic acid and protein in plasmid-containing minicells of Escherichia coli K-12, *J. Bacteriol.* 107 (1971), 21-33.
4. Rubenstein, K. E., Nass, M. M. and Cohen S. S.: Synthetic capabilities of plasmolyzed cells and spheroplasts of Escherichia coli, *J. Bacteriol.* 104 (1970), 443-452.
5. Levy, S. B.: Resistance of minicells to penic llin lysis: a method of obtaining large quantities of purified minicells, *J. Bacteriol.* 103 (1970), 836-839.
6. Sippel A., and Hartman, G.: Mode of action of rafamycln on the DNA polymerase reaction, *Biochim. Biophys. Acta* 157 (1968), 218-129.
7. Austin, S. J., Tittawella, I. P., Hayward, R. S., et al.: Amber mutations of Escherichia coli RNA polymerase, *Nature* (New Biol.) 232 (1971), 133-136.
8. Reichardt L., and Kaiser, A. D.: Control of lambda repressor synthesis, *Proc. Natl. Acad. Scl. U.S.A.* 68 (1971), 2185-2189).