DIMERS OF ESCHERICHIA COLI F' FACTORS†

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Summary: Covalently closed circular DNA dimers of several E. coli sex factors have been isolated. One of these, F'451, a dimer of F'450, has a molecular weight of ca. 230 x 10^6 daltons. F'451(λ) containing a λ prophage has a molecular weight of 260 x 10^6 and is probably the largest covalent closed circle of DNA yet reported. These dimers arise spontaneously and are of unknown origin and significance.

Plasmid-containing bacterial strains sometimes contain circular DNA dimers although they are relatively uncommon (1,2). In this paper we report the existence of dimers of some E. coli sex factors and the isolation of pure stable clones containing them.

E. coli strain B583 contains the sex factor, F'450, which includes the genes gal, att^{λ}, bio, and uvrB*† (3, 4). If cells are labeled with H^3-thymidine, lysed, and sedimented through in alkaline sucrose gradients, using the methods of Freifelder et al. (3), a single rapidly sedimenting peak consisting of covalent circles of F'450 is usually observed (Fig. 1a); on rare occasions

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† Reprints will not be available.

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*† Abbreviations: gal, ability to ferment galactose; att^{λ}, insertion site for prophage λ; bio, synthesis of biotin; uvrB, a locus involved in the repair of ultraviolet damage; lac, ability to ferment lactose; F'450(λ), F'450 containing a λ prophage.

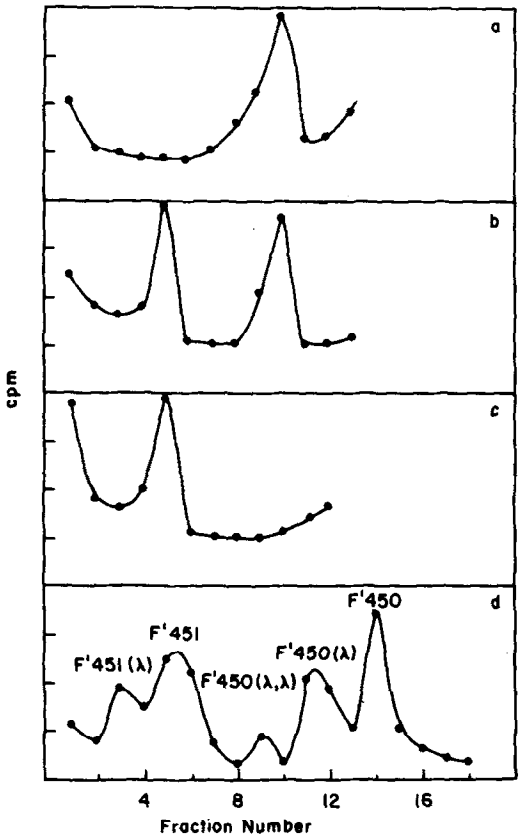

Figure 1: Sedimentation profile of lysates of various bacterial strains centrifuged in alkaline sucrose gradients. Cells were labeled with H^3-thymidine, concentrated, and lysed as described in (3). The lysate was then layered on an alkaline (0.3 M NaOH, 0.5 M NaCl, 0.002 M EDTA) 5-20% sucrose gradient and centrifuged for 29 minutes in a Beckman SW41 rotor at 38000 rpm (a) F'450; (b) F'450 containing 50% F'451; (c) F'451 clone; (d) mixture of F'450, F'450(λ), F'450(λλ), F'451, and F'451(λ). After centrifugation, gradient tubes were fractionated and the radioactivity of each fraction determined. In each case only a portion of the gradient is shown - the absent part containing the chromosomal DNA. In (d) the fractions were half the volume of those in (a-c). Direction of sedimentation - right to left.

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a small fast sedimenting peak is also seen. This strain had been maintained in our laboratory in Difco nutrient agar stab cultures at 4° C for four years, but for the past 2-1/2 years our strains have been maintained in 0.8% nutrient agar (soft agar) at room temperature - a method of storage which has been used successful-

ly for more than ten years in many laboratories and has been highly recommended. Some months after this change we noticed that the small fast sedimenting peak that had occasionally been observed was almost always present, although its magnitude depended on the particular stock culture, ranging from 10-75% of the circular material (Fig. 1b). This fast material was more shear-sensitive than the original F'450 circular DNA suggesting that it was larger.

A fast material was also seen in alkaline sucrose gradient profiles of other strains containing F, the F'lac of Jacob, Brenner and Cuzin (5) and the F'gal att$^{\lambda}$bio of Eisen, Siminovitch and Mohide (6). In these strains, however, it never exceeded 10% of the total circular material nor did it increase significantly by storage in soft agar.

When B583 stocks stored in soft agar were plated, some of the colonies were observed to be smaller than average; when these small clones were grown and labeled, and analyzed by sedimentation, they often contained only the fast material (Fig. 1c). Large colonies still contained the slowly sedimenting material.

Clones which contain only the fast material behave in every way like the original strain, i.e. they transfer gal and uvrB; they sometimes segregate gal-, uvrB- and att$^{\lambda}$-females, they can be lysogenized by phage λ, the lysogenized form undergoes zygotic induction, and insertion into the fast sedimenting sex factor can be observed by the appearance of faster sedimenting covalent circles (7). Furthermore, these clones are relatively stable in that female segregants appear at a rate no greater than with the

parent strain and upon continued growth for ca. 50 generations in liquid media, the slower moving peak does not reappear.

We have not attempted to clone strains containing F and F'lac, since these do not show small colonies and screening by sucrose gradient analysis is too tedious.

By cosedimentation with a mixture of F'450, F'450(λ), F'450(λ,λ), (Fig. 1d), one can estimate that the rapidly moving species (which we now call F'451) has a molecular weight ca. twice that of F'450. Furthermore, measurement of the activity of galactokinase (using the procedure of Perlman and Pastan (8) in cultures containing F'450 and F'451 grown for ca. ten generations in the presence of the gratuitous inducer, fucose, has shown that the enzyme level in a strain containing F'451 is twice that of a strain containing F'450. Since in both cases the chromosomal galactokinase gene is deleted, this supports the idea that F'451 is a dimer of F'450. To confirm this, a length measurement has been carried out by electron microscopy. Covalent circles were isolated by equilibrium centrifugation in CsCl containing ethidium bromide (9) from a clone containing the presumptive dimer. After removal of the dye by incubation with 50W-X2, cation exchange resin (Bio-Rad Labs.) the samples were prepared for electron microscopy using the formamide modification of the protein monolayer technique as described by Davis, Simon and Davidson (10). Covalently closed superhelical circles were found. We have not made a detailed study of the lengths of these molecules because of the difficulty in finding molecules whose length can be unambiguously measured. However, a few molecules were measured and

found to have an average length of 115 ± 5 microns indicating a molecular weight of roughly 230 x 10^6 daltons. Since F'450 has a molecular weight of 114 x 10^6 daltons (4), F'451 is probably a dimer of F'450 and is both the largest sex factor DNA and the largest superhelical circle yet reported. (We have also observed in alkaline sucrose gradients F'451(λ) covalent circles which would have a molecular weight of 260 x 10^6 daltons.) The ease with which F'451 can be isolated and studied by centrifugation in alkaline sucrose gradients suggests that with care even larger molecules could be studied. In fact it is likely that F. Hutchison (personal communication) at Yale University has handled non-superhelical circles of more than 2 x 10^9 daltons.

How F'451 dimers arise is not clear. The fact that they are enhanced in populations grown under very limited conditions (i.e. at room temperatures, very high cell density and probably with partial starvation for nutrients and O_2) agrees with a current proposal by R. Rownd (personal communication) that multimers of R factors appear when a culture is put under stress of some kind - in his case, in the presence of inhibiting antibiotics.

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