MOLECULAR NATURE OF AN IN VITRO RECOMBINANT MOLECULE: COLICIN E1 FACTOR CARRYING GENES FOR SYNTHESIS OF GUANINE

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Summary: The molecular nature of an in vitro recombinant DNA, which consists of colicin E1 DNA, a bacterial gene for xanthosine 5'-monophosphate aminase and a part of the λ phage genome, was studied by electron microscopy, restriction DNase digestion, and electrophoresis in agarose gel. This molecule existed as a monomer plasmid with an average molecular weight of 21.6 x 10^6 daltons within E. coli. The present results confirmed the unique structure of the molecule and its potential use as a cloning vehicle.

A recombinant molecule was constructed by in vitro combination of colicinogenic factor E1 (=ColE1) DNA and a guaA gene of E. coliK12 derived from λpguaA transducing phage[1, 2]. This molecule existed as a stable plasmid within E. coli and contained a whole ColE1 DNA and a gene for the guaA enzyme (=xanthosine 5'-monophosphate aminase) together with a part of the λ genome, R through J: (R-cos-A-F-J)$^+$ [2, 3]. Hereafter, this recombinant molecule is named ColE1-cosλ-guaA[3]. Fukumaki et al. found that ColE1-cosλ-guaA plasmids are efficiently packaged within λ phage particles by infecting λ phage onto (or, by inducing λ lysogens of) E. coli which carries these plasmids[3]. Their findings mean that the genetic properties of the recombinant plasmid can be studied by the convenient methods of λ phage

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Abbreviations: The genetic symbols are those used by Taylor and Trotter (4) for E. coli and by Szybalski and Herskowitz (5) for λ.

genetics. Because of this unique property and the potential usefulness of the molecule as a vehicle for gene engineering[3], we studied the molecular nature of this recombinant DNA by electron microscopy, restriction DNase digestion, and electrophoresis in agarose gel.

Materials and Methods

Bacterial strain: E. coliK12 KS1616 was isolated from HfrH and was deleted of a gal-attλ-bio region and a guaA-guaB region of a E. coli chromosome[2]. TM96 is derived from KS1616 and carries ColE1-cosλ-guaA plasmids[1].

Preparation of plasmid DNA: The ColE1-cosλ-guaA DNAs were accumulated by incubating TM96 in the presence of 100 μg/ml of chloramphenicol. Extrachromosomal DNA was extracted and purified as described previously[3, 6]. Purified covalently closed circular DNA was stored in TE buffer (100 mM Tris-HCl and 10 mM EDTA pH 7.5) at -10°C. ^{3}H-labeled ColE1-cosλ-guaA and λdv DNA were prepared as described previously[3, 6]. ColE1 DNA was a generous gift from Dr. T. Ogawa.

Measurement of DNA length by electron microscopy: The ColE1-cosλ-guaA DNAs stored in TE buffer at -10°C were lysed and mixed with ColE1 DNA and the molecules were photographed as described by Ogawa et al.[7], using a JEOL model 100C electron microscope. The length of the DNA strands in enlarged photographs was measured with a map measure (Maruzen Co., Tokyo). During storage at -10°C for about a month, approximately 30 % of the covalently closed DNA molecules were converted to open circular DNA molecules.

Enzymes: Restriction enzyme EcoRI was prepared from E. coli strain YR13, which was kindly supplied by Dr. R. Yoshimori[8]. Serratia marescens endonuclease R (SmaR) was purified as described by Tanaka & Weisblum[9]. An enzyme from Hemophilus influenzae, HindIII[10] and a restriction endonuclease from Bacillus amiloliquefaciens, BamI[11] were generous gifts from Drs. M. Takanami and T. Andoh, respectively. ATP-dependent DNase was purified from Micrococcus luteus as described by Anai et al.[12]. Digestions of purified plasmid DNAs by restriction enzymes and by ATP-dependent DNase were performed as described elsewhere[8-12].

Electrophoresis in agarose gel: The DNA fragments were subjected to agarose gel (0.8 %) electrophoresis[13]. The gel was stained with ethidium bromide and the fluorescent bands of DNA were photographed under long wavelength ultraviolet light.

Results and Discussion

The molecular weights of ColE1-cosλ-guaA DNA molecules were estimated by measuring their contour lengths in electron micrographs, relative to that of ColE1 DNA (4.2×10^6 daltons)[14]

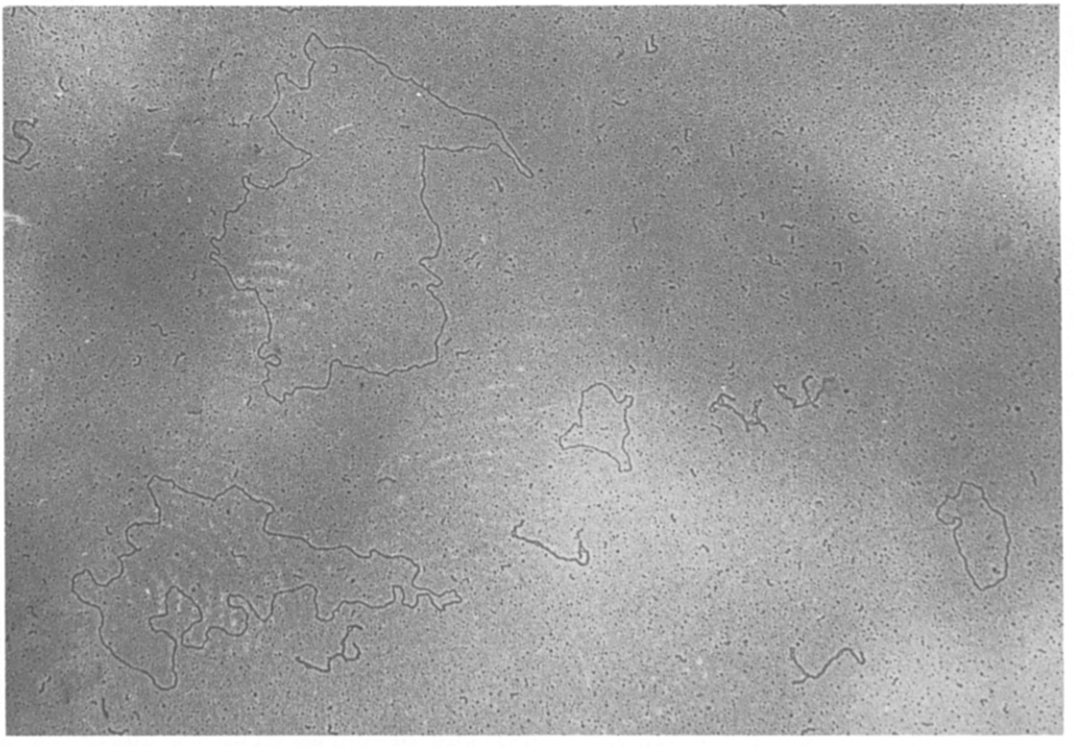

Fig. 1. Electron micrographs of open-circular ColE1-cosλ-guaA molecules. Small circular DNAs are those of reference ColE1.

(Fig. 1). The average molecular weight of fifty-two open circular DNA molecules examined was 21.6 ± 0.2 x 10^6 daltons. No apparent polymers were found among them.

As this recombinant molecule was constructed by *in vitro* combination of EcoRI-treated ColE1 and λpguaA phage DNAs, it is expected to have two EcoRI sites. In fact, EcoRI-treatment of the ColE1-cosλ-guaA plasmid produced two DNA fragments, e1 and e2 (Fig. 2c); e1 corresponds to the cosλ-guaA fragment of

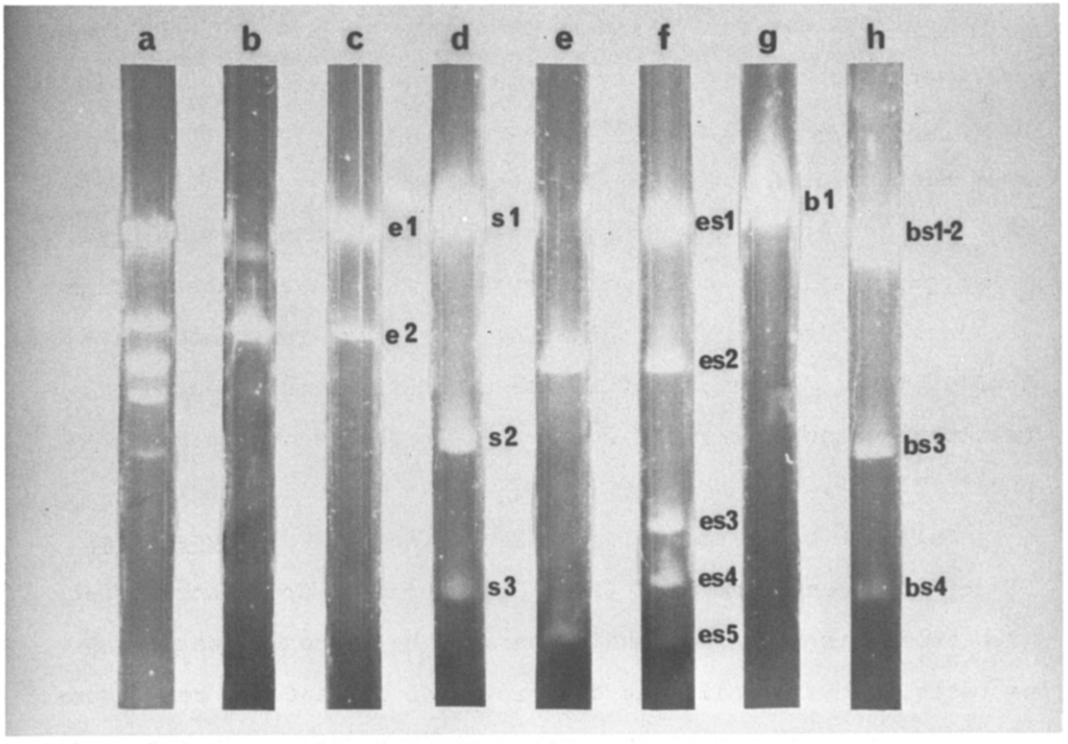

Fig. 2. Agarose gel electrophoresis of ColE1-cosλ-guaA plasmid DNA cleaved by restriction endonuclease. DNA fragments obtained after hydrolysis by EcoRI, SmaR, BamI, EcoRI + SmaR and BamI + SmaR are numbered with the prefixes e, s, b, es and bs, respectively. The molecular weight (x 10^6) of each DNA fragment was estimated using a reference curve constructed with EcoRI hydrolysates of λ phage DNA and with EcoRI or EcoRI + SmaR treated ColE1 DNA: e1, ca17.5; e2, 4.2; s1, ca18.5; s2, 2.0; s3, 1.0; es1, ca15.0; es2, 3.5; es3, 1.3; es4, 1.0; es5, 0.7; b, ca20.0; bs1-2; 9.2; bs3, 2.0; bs4, 1.0. (a) λcI857 DNA + EcoRI, (b) ColE1 DNA + EcoRI, (c) ColE1-cosλ-guaA DNA + EcoRI, (d) ColE1-cosλ-guaA DNA + SmaR, (e) ColE1 DNA + EcoRI & SmaR, (f) ColE1-cosλ-guaA DNA + EcoRI & SmaR, (g) ColE1-cosλ-guaA DNA + BamI, and (h) ColE1-cosλ-guaA DNA + BamI & SmaR.

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the molecule and e2 to linear ColE1 DNA[1]. When these plasmid DNAs were treated with other restriction enzymes, such as SmaR, BamI or HindIII, and analyzed by agarose gel electrophoresis,

it was found that they had three SmaR sites, only one BamI site and no HindIII site.

The approximate location of the targets for SmaR in ColE1-cosλ-guaA DNA was determined by comparing the sizes of DNA fragments obtained after hydrolysis with SmaR and after hydrolysis with SmaR plus EcoRI. SmaR treatment of this recombinant molecule produced three DNA fragments; s1, s2 and s3 (Fig. 2d). The five fragments, es1 to es5 appeared on treatment with SmaR and EcoRI (Fig. 2f). The fragments s1 and s2 were absent from the double hydrolysates, indicating that these fragments have EcoRI sites. The sizes of all these DNA fragments and their relation to each other are summarized in the legend to Fig. 2 and in Fig. 3.

Only one component, b1, was identified in ColE1-cosλ-guaA DNA by treatment with BamI (Fig. 2g), and four fragments, bs1-2 (two fragments which were not separated by agarose electrophoresis, see the following description), bs3 and bs4 were identified by treatment with BamI and SmaR (Fig. 2h). In the double hydrolysates, fragment s1 was not seen and one new fragment bs1-2 of about half the size of s1 appeared (Fig. 2h), indicating that fragment bs1-2 is composed of two fragments of approximately equal molecular size (Fig. 3). Recently, Perricaudet & Tiollais reported the presence of one BamI susceptible site within the λ head gene E[15]. Our results are in good agreement with their finding.

The electrophoretic mobility of the ColE1-cosλ-guaA DNA preparation was not changed by treatment with HindIII (data not shown), suggesting the absence of a site susceptible to HindIII in this molecule. We confirmed this using ATP-dependent DNase, which can degrade only linear DNA molecules[16]. ^{3}H-labeled

closed circular ColE1-cosλ-guaA DNA and λdv DNA were prepared[6], treated with HindIII and further digested by ATP-dependent DNase. λdv DNA is known to have at least one site susceptible to HindIII[17]. About 98 % of ^{3}H-labeled λdv DNA was converted to acid-soluble materials by this treatment. On the other hand, only 1.7 % of ColE1-cosλ-guaA DNA became acid-soluble on the same treatment.

It seems probable from the above results that the locations of restriction endonuclease-susceptible sites on ColE1-cosλ-guaA are as shown in Fig. 3. The sum of the molecular weights of the fragments from ColE1-cosλ-guaA, treated with various restriction enzymes (see legend to Fig. 2), was about 21.5 x 10^{6}, which agrees with that of the whole molecule measured from electron micrographs. From these results, we conclude that

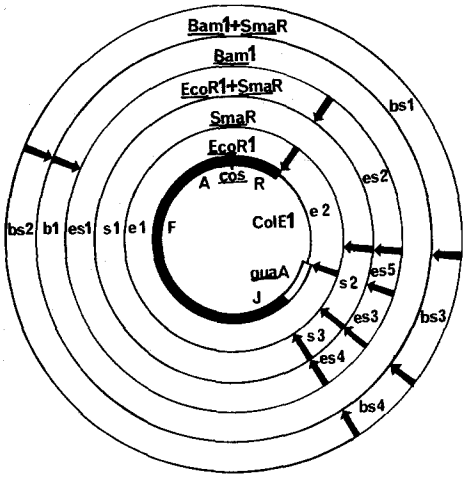

Fig. 3. Sites of cleavage of ColE1-cosλ-guaA DNA by several restriction endonucleases. The thin line represents whole ColE1 DNA, the thick line a part of the λ genome and the double line a fragment of the bacterial chromosome. The drawing is approximately to scale. A, F, J, R and cos are λ phage gene markers[5], guaA is a bacterial gene marker[4] and ColE1 indicates colicinogenic factor E1 DNA. Arrows indicate positions of sites susceptible to the restriction endonucleases. Other symbols are as for Fig. 2.

most of the recombinant molecules are present as monomers within E. coli cells.

As we found that ColE1-cosλ-guaA DNA has only one BamI sensitive site, various hybrid molecules could be constructed in vitro by introducing a BamI-digested foreign DNA fragment into BamI-cleaved ColE1-cosλ-guaA plasmid DNA. The λ phage head can package 109 % of the λDNA length (about 35 x 10^6 daltons) into its head without losing any plaque-forming ability[18]. When a genetically uncharacterized foreign DNA fragment is connected with ColE1-cosλ-guaA DNA, it can exist as a part of stable plasmid DNA. If the foreign DNA introduced is less than 13 x 10^6 daltons in size, this recombinant molecule can be packaged within a λ phage particle, and studied by the methods of λ phage genetics.

When various specialized transducing λ phages are infected onto TM96, the lysogens most probably carry λDNAs within ColE1-cosλ-guaA plasmids, because this strain is deleted of the normal λ attachment site[2], and because there is much homology between λ DNA and ColE1-cosλ-guaA plasmid DNA, which carries a block of the λ genome R through J (Fig. 3)[3]. Various in vivo recombinant ColE1 plasmids have been isolated after induction of these lysogens (Shimada et al. in preparation). The cleavage map of the ColE1-cosλ-guaA molecule should be of value in elucidating the genetic structure of these new plasmids.

The efficient in vivo packaging of a monomer plasmid DNA with only one cosλ site by λ phage is another interesting problem[3, 19]. The recombinant molecule studied in this work should be a useful substrate in this study.

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