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Physical characterization of the *E. coli dnaC* region carried by a plaque forming *λdnaC* transducing phage

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Summary. The physical map of the 11.5 kb DNA segment containing the *E. coli dnaC* gene carried by λp*dnaC* transducing phage was constructed and the *dnaC* gene within this segment was localized by subcloning it into plasmid pBR322. Based on the physical structure of λp*dnaC*, the formation of the λp*dnaC* by nonhomologous recombination is dicussed.

Among the proteins required for DNA replication in *E. coli*, the *dnaC* gene product is particularly interesting because it is needed for both initiation and elongation processes[2,3]. Previously, we had isolated 2 plaque forming λ phage derivatives transducing the *dnaC* gene, λp*dnaC*-17 and λp*dnaC*-37, through a) directed integrative suppression of a *dnaA* mutation by an F′ plasmid carrying *dnaC*, b) isolation of λd*dnaC* and c) conversion of λd*dnaC* into λp*dnaC* with the help of λ*imm*21*b*2*nin*5 prophage[4]. We describe here the physical map of λp*dnaC*, which may serve as a basis for functional studies.

The structures of λp*dnaC*-17 and λp*dnaC*-37 are identical since they produced the same restriction cleavage patterns (data not shown). According to the procedure for the isolation and genetic characterization of λp*dnaC*[4], its genotype must be λ*imm*21*nin*5*int*$^+$*attP*$^+$*dnaC*$^+$. Indeed, electron microscopic heteroduplex studies (fig. 1,A) showed that it carries the *imm*21 substitution, the *nin*5 deletion and an 11.3±0.7 kb *dnaC* substitution (19 molecules analyzed). The *dnaC* substitution begins at λ map unit[5] 0.45 and terminates at λ map unit 0.57. This extension corresponds very closely to that of the *b*2 deletion[5], which covers 5.76 kb between λ map units 0.453 and 0.573. We have also analyzed λp*dnaC* DNA with restriction enzymes (fig. 1,C–F) and constructed a restriction cleavage map (fig. 2). The results confirmed that the *dnaC* segment replaced the *b*2 region of the λ genome. The *Bam*HI and *Hin*dIII sites[5] on the λ genome at λ map units 0.466 and 0.568, respectively, are lost due to the substitution, but the *Eco*RI site[5] at map unit 0.445 is still present. Three *Eco*RI sites[5] carried on wild type λ DNA at map units 0.653, 0.810 and 0.931 are absent because the *att*P-*int*-*imm*21-*nin*5-*R* region of λp*dnaC* originated from the λ*imm*21p*lac*5*nin*5 derivative λ616[4,6], on which these *Eco*RI sites are missing (see also fig. 3). From these analyses we conclude that the approximately 11.5 kb *dnaC* segment contains 2 *Eco*RI sites, 2 *Bam*HI sites, 1 *Bgl*II site and 2 *Hin*dIII sites. These are shown in figure 2.

To localize the *dnaC* gene further, λp*dnaC* DNA was cleaved with *Eco*RI, mixed with *Eco*RI cleaved pBR322[7], ligated and transformed to *E. coli* C LD332 *dnaC*ts[4] with selection for temperature resistant (tr), ampicillin resistant (Ap^r), and tetracycline resistant (Tc^r) transformants. Isolation and restriction cleavage analysis of plasmids[8] from these transformants revealed that they were pBR322 derivatives containing the 8.3 kb *Eco*RI-3 fragment (see figs 1 and 2). Similarly, pBR322 containing the 4.7 kb *Bam*HI-5 fragment inserted in its *Bam*HI site gave tr, Ap^r, Tc^s transformants of LD332. That production of tr transformants is not dependent on recombination of the *dnaC* segment into the host chromosome was shown by obtaining tr, Ap^r, Tc^s transformants of *E. coli* K12 NY60 *dnaC*ts *recA*[2] with appropriately (*Eco*K) modified pBR322:*Bam*HI-5 plasmid. Growth or tr transformants is thus rendered possible by complementation. Since the orientation of the *Bam*HI-5 fragment inserted into pBR322 did not affect

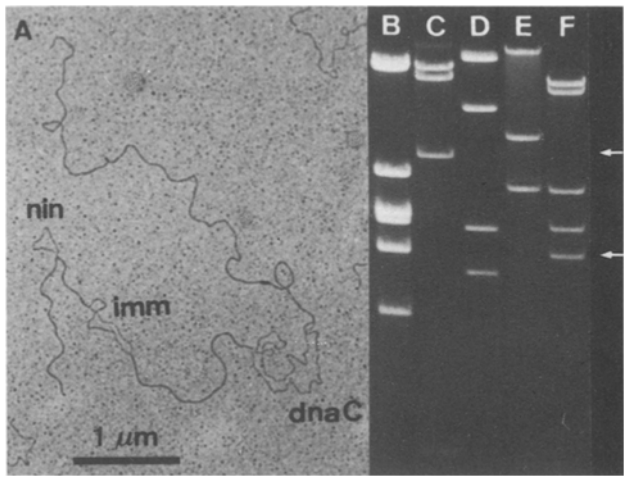

Figure 1. Heteroduplex molecules between λp*dnaC* and λ DNA, and restriction cleavage patterns of DNA of λ and λp*dnaC*. Preparation of λ phages, formation of heteroduplex molecules, extraction of phage DNA and restriction cleavage analysis were performed as described before[4,9]. *A* Heteroduplex between DNA of λp*dnaC* and λ*cI*857*S*7. Substitution loops for the immunity region (*imm*) and for the *dnaC* segment and the *nin*5 deletion loop are marked. *B* to *F* Restriction cleavage patterns of λ DNA and λp*dnaC*. Electrophoresis was carried out in a 0.8% Agarose gel. *B* λ*cI*857*S*7 DNA cleaved with *Eco*RI; *C* λp*dnaC* with *Eco*RI; *D* λp*dnaC* with *Hin*dIII; *E* λp*dnaC* with *Bgl*II; *F* λp*dnaC* with *Bam*HI. The arrows point to the *Eco*RI-3 and *Bam*HI-5 fragments, both of which contain the *dnaC* gene.

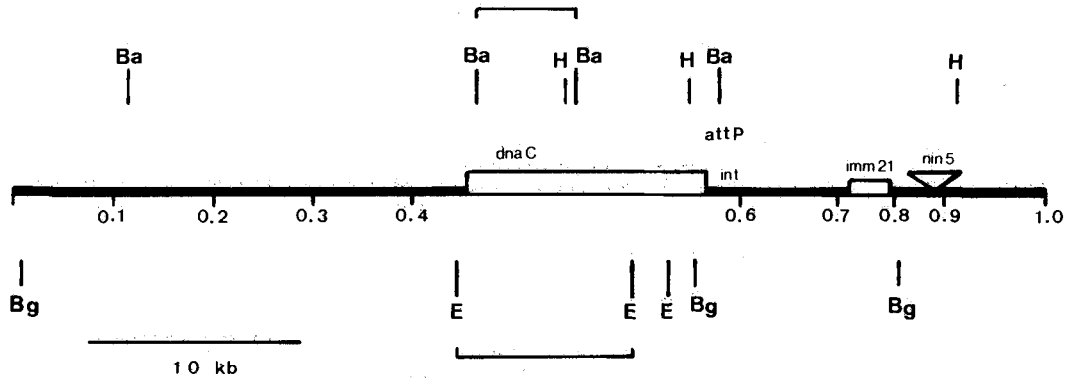

Figure 2. Restriction cleavage map of λp*dnaC*. The bacterial DNA is represented by a large box, the λ genome by a solid bar. The coordinates are given in λ map units[5]. The small clear box represents the *imm*21 substitution and the open triangle the *nin*5 deletion. The methods used to construct the restriction cleavage map were described previously[10]. Restriction cleavage sites are: Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII. The brackets above and under the map indicate the *dnaC* containing *Bam*HI-5 and *Eco*RI-3 fragments, which were subcloned into pBR322.

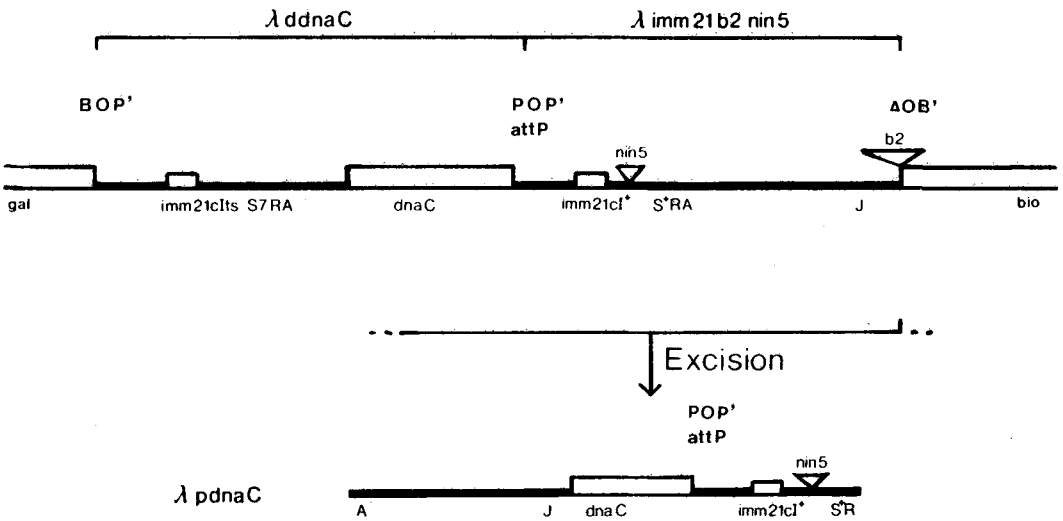

Figure 3. Formation of λp*dnaC* from a double lysogen for λd*dnaC* and λ*imm*21*b*2*nin*5. Construction and genetic characterization of the double lysogen and of λp*dnaC* phages were described previously[4]. Physical characterization of the λp*dnaC* DNA now suggests that this phage must have resulted from excision by nonhomologous recombination between a site at or near *Δ*OB′ and a site in the *dnaC* segment of λd*dnaC*. However, the exact crossover sites in this excision have not been mapped. The map is drawn in the same way as in figure 2. The *att*P-*imm*21-*nin*5-*R* region of λ*imm*21*b*2*nin*5 originated from λ616 which had lost three *Eco*RI sites[4,6].

dnaC complementation (data not shown), this *Bam*HI fragment must contain the promotor for the *dnaC* gene. In addition, a spontaneous deletion derivative of the pBR322:*Bam*HI-5 plasmid having lost the *Hin*dIII site also complemented a *dnaC*ts mutation, suggesting that the *dnaC* gene does not locate at the very right end of the *Bam*HI-5 fragment as shown in figure 2. From these results, the *dnaC* gene can be mapped within 4.4 kb on the *Bam*HI-5 fragment of λp*dnaC*. Note that the *dnaC* gene is expected to occupy about 1 kb of DNA because the molecular weight of the *dnaC* gene product[3] is 29,000.

The plaque forming transducing phage λp*dnaC* had been obtained from a double lysogen for λd*dnaC* and λ*imm*21*b*2-*nin*5[4]. The revealed structure of λp*dnaC* (fig. 2) suggests that λp*dnaC* is likely to be formed by nonhomologous recombination between the *dnaC* segment of λd*dnaC* and either the *Δ*OB′ site or the adjacent sequence of the *E. coli* chromosome (fig. 3). The absence of three *Eco*RI sites on λp*dnaC* (fig. 2) is consistent with this interpretation.

After this work had been completed, we learned that Kornberg and his collaborators also have characterized the λp*dnaC*-17 DNA (A. Kornberg, personal communication). Their result on the localization of the *dnaC* gene on λp*dnaC* agrees with ours.

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