Short Communication

Construction of "Sleepers" Cloning Vehicles for Enzyme Overproduction

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Molecular cloning is a powerful means of producing specific gene products.^{1~4)} High yields of gene products are usually achieved by using cloning vehicles capable of copy number change. The number of copies of such vehicles can be increased conveniently by means of thermal induction. Phage λ vehicles with a temperature-sensitive *cI* gene product are reported to be useful for convenient amplification.^{1,3)} Plasmid vehicles derived from a so-called runaway-replication mutant have also been reported to replicate without copy number control above 35°C.⁴⁾ In this communication, we report the construction and partial characterisation of a new series of amplifiable vehicles named "Sleepers" vehicles.

As shown in Fig. 1, the "Sleepers" vehicles presented are hybrid lambdoid phages between λ CI₈₅₇ and ϕ 80, whose DNA has *Eco*RI cleavage sites only on the left half of the DNA

FIG. 1. Map Locations of *Eco*RI Cleavage Sites in the Genome of λ CI₈₅₇, ϕ 80, and "Sleepers" Vehicles (slp1, slp2, slp1S, and slp2S).

Solid bars indicate DNA derived from λ ; and open bars, ϕ 80. *Eco*RI fragments are numbered below the bars. Some genetic markers are lettered above the bars.

molecule. Since the genetic information for phage coat proteins is located on the left half of the DNA molecule in lambdoid phages,⁵⁾ foreign DNA fragments to be cloned replace most of the DNA regions for the production of phage coat proteins in our vehicles instead of non-essential DNA regions of earlier λ vehicles.^{6,7)} The recombinant DNA molecule thus constructed loses the ability to produce phage particles in a host cell, and can be maintained in a lysogenic state on a host chromosome. Incubation of the lysogenised cells at high temperatures induces replication of the recombinant DNA molecule, resulting in amplification of the inserted genetic information, while in the lysogenic state, the expression of the inserted genes is not so high as in the case of cloning with plasmid vehicles, therefore, the genes cloned are maintained stably on a "Sleepers" vehicle.

To construct these "Sleepers" vehicles, a deletion mutant, λ CI₈₅₇b6042, was isolated from λ CI₈₅₇ according to the method of Parkinson and Huskey.⁸⁾ Three *Eco*RI cleavage sites on this DNA were eliminated by the method of Murray and Murray,⁹⁾ and a λ CI₈₅₇b6042/*Eco*RI phage whose DNA has no *Eco*RI cleavage site was isolated. λ DNA has no *Eco*RI site on the left half of the molecule (Fig. 1), therefore ϕ 80 was crossed with λ CI₈₅₇b6042/*Eco*RI to obtain a λ CI₈₅₇h80att⁸⁰ slp2 vehicle. Since λ CI₈₅₇b6042/*Eco*RI deletes the central region of the DNA molecule where the genes needed for lysogenisation are located, λ^+ phage was crossed to recover this region. λ CI₈₅₇12-2 thus obtained has a DNA molecule of normal size with two *Eco*RI sites on the left side of *att*. In order to construct a vehicle with *att^L*, λ CI₈₅₇12-2 was crossed with ϕ 80*ptrp*, which was used instead of ϕ 80 so as to prevent frequent crossover of ϕ 80 with λ on the right side region of *att⁸⁰*, and thus a λ CI₈₅₇h80att^L slp1 vehicle was obtained.

Slp1S and slp2S vehicles which are *Sam* mutants and therefore can not lyse a non-permissive host upon infection, were constructed by recombination between λ CI₈₅₇b6042*Sam*₇/*Eco*RI and a clear mutant of

slpl or slp2. The $\lambda cI_{857}b6042Sam_7/EcoRI$ was isolated from $\lambda cI_{857}b6042Sam_7$ which had been constructed from $\lambda cI_{857}h80Sam_7$ and $\lambda cb6042/EcoRI$.

Since the insertion of foreign DNA fragments into the "Sleeper" vehicle by *in vitro* recombination leads to the formation of recombinant molecules without most of the genes needed for the synthesis of phage coat proteins, the recombinants can not be isolated by the methods employed for the earlier λ vehicles^{6,7)} which involve the infection of recombinant DNA into the competent cells¹⁰⁾ followed by "plaque" formation on an appropriate selection medium. An *EcoRI* DNA fragment of 13.4×10^6 daltons bearing *E. coli trp* genes was isolated by cloning with a pBR325 plasmid vehicle.¹¹⁾ The *trp* DNA fragment was mixed with slpIS DNA cleaved with *EcoRI*, and then treated with T4 DNA ligase. The recombinant DNA was incorporated into *E. coli* 1100*Atrp*(slpl) cells by the method of Kaiser and Hogness,¹²⁾ and *trp*⁺ transductants were isolated. In order to cure the slpl phage genome, these were incubated at 43°C for 30 min, and then plated on a minimal medium lacking tryptophan. Colonies on this plate were isolated and examined for active phage production. Thus, *E. coli* 1100*Atrp*(slplS-*trp*) was obtained.

Several reports have been presented on the amplification of enzyme synthesis with λ transducing phages constructed *in vivo*¹³⁾ or *in vitro*.^{1,3,14,15)} In order to examine the extent of enzyme synthesis, *E. coli* 1100*Atrp*(slplS-*trp*) was cultured in T-Y medium (1% tryptone, 0.5% yeast extract, and 0.5% NaCl) at 33°C with shaking. The growth was followed with a Klett-Summerson colorimeter and when the Klett unit with a No. 66 filter reached 30, the cultural temperature was adjusted to 43°C for 15 min and then to 37°C for 4 hr. Cells were collected by centrifugation, sonicated, and then subjected to the assay for tryptophan synthetase activity as described by Smith and Yanofsky.¹⁶⁾ As a control without thermal induction, the *E. coli* lysogen was cultured at 33°C until the Klett unit reached

TABLE I. OVERPRODUCTION OF TRYPTOPHAN SYNTHETASE BY *E. coli* 1100*Atrp*(slplS-*trp*)

Strain No.	Thermal induction	Growth (Klett units)	TSase* (units/liter)
1128	—	108	9
1128	+	123	16900
215	—	110	14
215	+	111	45900

* TSase, tryptophan synthetase activity. One unit causes the disappearance of 0.1 μ mol indol in 20 min at 37°C.

110. A 2,000 to 3,000-fold increase in tryptophan synthetase activity was observed as shown in Table I upon thermal induction as compared to the activity of a control with no induction. Panasenko *et al.*¹⁾ reported that lysogens containing λ gt-*lop*-11 *lig*⁺ *S*₇, a plaque forming transducing phage constructed *in vitro*, produce a 500-fold increase in DNA ligase activity upon thermal induction as compared with that in uninfected *lig*⁺ cells. Since the *lop*-11 mutation itself, however, leads to a 5-fold overproduction of ligase,¹⁾ the increase by induction may be about 100-fold. As to the plasmid vehicles capable of runaway replication, Uhlin *et al.*⁴⁾ reported that β -lactamase activity could be amplified up to 400-fold after thermal induction of the runaway replication as compared also with that in the original strain from which the β -lactamase gene had been cloned.

In general, plasmids are known to be frequently lost from their hosts during long-time storage on medium. As for the lysogens of the recombinants constructed *in vitro* with "Sleeper" vehicles, they could be stored for at least 6 months at room temperature without detectable loss of the cloned genes. During the course of curing experiments of the lysogen, it was observed that old cells are considerably less susceptible to thermal induction than the log-phase cells. This fact supports the stability described above. Thus, "Sleeper" vehicles seem to be excellent for preservation of cloned genes as well as for amplification of enzyme synthesis and, therefore, they are suitable for industrial use. Further detailed studies are in

progress and will be reported elsewhere.

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