CONJUGAL TRANSFER OF PLASMID λdv

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SUMMARY: Plasmid λdv,originating from a genome of phage λ was found to be mobilized with self-transmissible plasmids, such as F'lac, ColIdrd or R100-1. The frequency of transfer (10^{-4} - 10^{-5}) was similar to that for an another non-self-transmissible plasmids, pSC122, but was lower than that (10^{-0}) observed with yet an another non-self-transmissible plasmid, ColE1 factor.

In donor cells, λdv are perpetuated as dimers. However, in many cells receiving the mobilized λdv, the plasmid DNA was found in monomeric form.

Plasmids which replicate autonomously in bacterial cells in an extra-chromosomal state are classified into two types (1): Those which are self-transmissible, such as F, ColI factor and some R factors, and those which are non-self-transmissible, such as ColE1 factor, $mini_{15}$, λdv and pSC101 and their derivatives (for a review of these plasmids, see ref. 1).

Several naturally-occurring plasmids of the latter type have been observed to be mobilized with a coexisting self-transmissible "sex factor", and has been seriously considered in construction of safe vehicles for gene cloning experiments (2). The mobilization does not seem to be associated with a direct, stable recombinational union between the sex factor and the plasmid. However, the frequency of mobilization varies depending upon combinations of two types of plasmids (3 - 8). In this respect, it was of interest to examine whether λdv, which is used as an another cloning vehicle (9), would also be mobilized by the sex factor. This plasmid is a one-tenth fragment of the bacteriophage lambda genome, existing in dimeric form and about 60 copies are perpetuated per carrier chromosome (10,11). The result shows that λdv is transferred with F or R100-1, or ColIdrd. Reduction in size of λdv from dimeric to monomeric form occurs during the mobilization process.

MATERIALS AND METHODS

Bacteria: All the bacterial strains are derivatives of Escherichia coli K12. TM43 (str,his,recA1,gal^{del},lac) is a lac derivative of KM723 (10). This strain,

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Table 1. Plasmids used

Plasmid	Marker and Special Properties	Incompatibility Group	Reference or Source
λdv1	immune to superinfecting λ phage: normally exists in dimeric form	--	K. Matsubara (10,11)
λdvkan1	λdv carrying a kanamycin^R transposon in the cI region	--	D. Berg
pSC122	a derivative of pSC101 (penicillin^R, tetracycline^R): non-self-transmissible	--	S. N. Cohen (13)
F'lac	Lac$^+$ derivative of an F sex factor	FI	F. Jacob
R100-1	a derepressed transfer mutant of R100: chloramphenicol^R, tetracycline^R sulfonamide^R	FII	Y. Hirota
ColIdrd	a derepressed transfer mutant of ColI factor: produce colicin I	Iα	H. Ozeki
N3	a self-transmissible drug-resistant plasmid: tetracycline^R, sulfonamide^R, streptomycine^R	N	T. Arai
RP4tnC261	a self-transmissible drug-resistant plasmid: tetracycline^R, ampicillin^R	P	P. Barth

Donor cells were constructed first by transforming TM43 with either λdv or pSC122 (10,13), followed by mating with appropriate cells to introduce a sex factor, such as F'lac, R100-1 etc. ColE1 factor was transferred by a cotransfer (3).

carrying both transmissible and non-transmissible plasmids was used as a donor. TM42 is a nal^R derivative of the TM43, and was used as a recipient. Hfr KL16-99 is a recA1 derivative of an Hfr that injects chromosome in an order: thy-recA-his, and was obtained from Dr. B. Low (12). The plasmids used are listed in Table 1.
Media: C broth contained, per liter: 4.2g K_2HPO_4, 1g NH_4Cl, 10g bactotryptone, 10g NaCl, 5g yeast extract, 5g glycerol, 100mg $MgSO_4$, 5mg $CaCl_2$, and 0.2mg $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 6.3 with NaOH (14). Nutrient agar was the one described as PBB agar (10).
Conjugal transfer experiments: Donor (nalidixic acid-sensitive) and recipient (nalidixic acid-resistant) cells were separately grown in C broth to a cell density of 1 x 10^8 per ml, mixed (1 donor:10 recipient) and incubated in a reciprocal shaking incubator for an indicated period at 37 C. When a culture of ColE1 or ColIdrd carriers was used, it was treated with trypsin (200ug/ml) prior to the mating. Cells that received λdv1 were selected by adding 1 x 10^7 particles each of $\lambda cI_{90}nin_5$ and $\lambda h^{80}imm^{\lambda}cI_{90}nin_5$ (10) to one ml of the mating mixture, and then poured over a nutrient agar plate supplemented with 20ug/ml

of nalidixic acid, and dried. After incubation for 36hrs at 37 C, cells that received λdv appeared as λ-tolerant colonies whereas all other cells were lysed by the phage (15). Cells that received λdvkanl or pSC122 were selected as kanamycin (20ug/ml) or penicillin (200U/ml) and nalidixic acid (20ug/ml) resistanct colonies. Cells which received colicin factor (ColE1 or ColIdrd) were scored by spreading appropriately diluted exconjugants over a nutrient agar plate containing nalidixic acid and then counting those colonies that exhibited inhibition of growth of C600S cells overlayed and incubated at 43 C. Cells that received F'lac were scored as red colony-formers on MacConkey agar supplemented with lactose and nalidixic acid. Cells that received R100-1, N3 or RP4tnC261 were selected as tetracycline (8ug/ml) and nalidixic acid-resistant colonies. The frequency of transfer was expressed as the number of cells that received the plasmid per donor cell present at the time of initiation of mating.

Sucrose density gradient centrifugation: DNA samples were prepared and analyzed by sedimenting through sucrose gradient (5 - 20%) containing 0.02M Tris-HCl, 2mM EDTA and 1M NaCl (pH 7.4), as described previously (10).

Cross-streak test: Colonies that carry λdv were picked by sterile tooth-picks and cross-streaked across a line of λ phage (10^9 λvir per ml) as described (15). Clones carrying dimeric and monomeric λdvl were distinguished with this test, because the dimeric λdvl carriers were completely tolerant against the phage and grew confluently where the two streaks overlapped, but clones carrying a monomeric λdvl were less tolerant and resulted in thin growth at the intersection.

RESULTS AND DISCUSSION: Plasmid λdv does not promote its own conjugal transfer. However, a coexisting sex factor, such as F'lac or R100-1, mobilized λdv at a demonstrable frequency, as shown in Table 2. F^+ and F'gal behaved similary (data not shown). The efficiency of mobilization of some non-self-transmissible plasmids is reported to differ depending upon difference in the sex factors (3,8). Table 2 shows that λdvkanl, that carries a kanamycin-resistant marker and thus allows detection of transfer with ease, is transferred also with ColIdrd, a member in the Iα incompatibility group, which utilizes different sex pili from that of the F or R100-1. Other sex factors including N3 and RP4tnC261 that belong to yet other incompatibility groups and that utilize different transfer machineries (17,18) were also tested, but transfer frequency of the sex factors themselves were low, and the mobilization of λdvkanl was too low to be detected.

The transfer frequency of other plasmids, ColE1 factor and pSC122 was compared and the results are shown in Table 3. ColE1 factor was mobilized by F'lac at high frequency as observed previously (3). The transfer frequency of pSC122 was similar to, or slightly lower than that of λdv. These values appear to be similar to the mobilization frequencies of other non-conjugative plasmids, such as N-SuSm and N-Tc (5), although direct comparison of the efficiencies is difficult because of the difference in mating conditions. At present, it is not clear why only ColE1 factor is mobilized at high efficiency. It could be that the ColE1 factor possesses a component(s) similar

Table 2. Mobilization of λdv1 or λdvkan plasmid with various sex factors

		Frequency of Transfer	
		λdv	Sex Factor
Exp. A			
λdv1		$< 3.3 \times 10^{-7}$	
λdv1	F'lac	4.4×10^{-4}	6.5
λdv1	R100-1	4.3×10^{-4}	10.2
λdv1	Hfr	$< 9.0 \times 10^{-8}$	0.44[a]
Exp. B			
λdvkan		$< 1.0 \times 10^{-8}$	
λdvkan	F'gal	3.3×10^{-3}	2.7
λdvkan	R100-1	2.3×10^{-4}	0.44
λdvkan	ColIdrd	2.0×10^{-5}	0.86
λdvkan	N3	$< 4.0 \times 10^{-8}$	4×10^{-2}
λdvkan	RP4tnC261	$< 1.0 \times 10^{-7}$	2.9×10^{-4}

Donor cells (TM43, Nal^S) carrying λdv1 (in dimeric form) or λdvkan1 and the indicated sex factor were grown into log phase, and mated with a recipient strain TM42 (Nal^R) at 37 C for 120min (Exp. A) or 90min (Exp. B). Cells receiving the plasmids were scored as described in Materials and Methods. The high frequency of transfer of sex factors may be due to the rather long mating period that would have resulted in secondary transfer from the primary zygote cells.

a) Because the recipient (TM42) was recA⁻, the ability of the Hfr strain to mobilize chromosomal markers was not directly tested. This value was inferred from an experiment run in parallel using KS143 (W3623 F⁻, trp,gal,thy,str) as a recipient and measuring the transfer of thy^+ marker.

to or common with that in the sex factor (16).

Transfer of λdv was not detectable when an Hfr strain (HfrKL16-99) was employed, though proximal chromosomal marker (Thy^+) was transferred at high frequency in 2hr mating. In an another experiment matings were done for 24hrs, without any detectable transfer of λdv. However, ColE1 factor is mobilized even with the Hfr strain as reported previously (3). It is not clear at present whether λdv is not mobilized by the Hfr at all or it is mobilized but the frequency is too low for detection.

In order to test whether or not the λdv mobilized by F has the same genetic composition as that in donor cells, marker rescue experiments were carried out (11). It was observed that λdv's before and after transfer have

Table 3. Comparison of cotransfer frequencies with λdv1 in dimeric and monomeric form, ColE1 factor and pSC122

Plasmids in The Donor		Frequency of Plasmid Transfer
λdv1(D)	F'lac	4.3×10^{-4}
λdv1(M)	F'lac	5.6×10^{-4}
ColE1	F'lac	1.58
pSC122	F'lac	6.1×10^{-5}

Matings and scoring number of cells receiving a mobilized plasmid were done as described in Methods. λdv1(D) or λdv1(M) represents, respectively, λdv1 in dimeric or in monomeric form.

the identical genetic constitution. A possibility of mistakingly selecting newly arisen λdv's from λcI_{90} phage (15) used in the process of selecting clones that received mobilized λdv was ruled out, since such plasmids were expected to lack $v_2v_1v_3$ markers (15), whereas all the λdv's in cells in question carried these markers.

Measurements of size of λdv DNA before and after the mobilization gave unexpected results: When these plasmid DNA's were sedimented in sucrose density gradients, over 99,95% of λdv DNA's in donor cells TM43(λdv1, F'lac) consisted dimeric molecules. In contrast to this, among twelve clones that had received the mobilized λdv's, six were found as carriers of λdv plasmids in monomeric form. DNA preparations from each of these clones invariably had a small amount (ca. 5%) of dimeric DNA, as has been observed with other monomeric λdv carriers descibed previously (10). The proportion of dimeric DNA in a population increased upon repeated dilution and culturing of the monomeric λdv carriers. Three clones yielded λdv DNA consisting of an equal amount of monomers and dimers, and the remaining three clones carried dimeric DNA only. Clones that contained the mixed λdv's became carriers of only dimeric λdv's after 25 more generations. The population drift in a monomeric λdv carrier culture to dimeric λdv carriers has been observed previously (10), possibly because the latter carriers have some growth advantage over the former carriers. It is likely that, in the intermixed clones, monomeric λdv would have been first appeared upon conjugal transfer. Other three clones that carried only dimeric λdv's could have been derived similarly, though in these cases direct proof was missing. In an another experiment using cross-streak tests in which tolerance to superinfecting λ was used as a probe to

discriminate monomer- and dimer-carriers (10), about 90% (114/125) of the cells that had received mobilized λdv carried monomeric λdv.

In order to test a possibility that the recipient cell (TM42) converted dimeric λdv into monomeric form, cells treated with $CaCl_2$ were transformed to λdv carriers by exposing to dimeric λdv DNA (10). All (80/80) the transformants tested were found as carriers of dimeric λdv, indicating that the specific conversion from dimers to monomers as observed in conjugal transfer was not the case in transformation. Moreover, there was no selective advantage or disadvantage for the mobilization from monomeric λdv carriers as shown in Table 3.

Apparently, in the cross using dimeric λdv carrier donor, the monomeric λdv must have been derived in association with the conjugal transfer process. The mechanism that produced the monomeric λdv is not clear at present. Since both donor and recipient cells used in these experiments were recA derivatives, a recA function of host cell did not play a role in this process.

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