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Specialized Transduction of D-Serine Deaminase Genes: Formation of Lysogens That Yield High λ-d *dsd*/λ Ratios and Formation of a Dimeric λ-d *dsd*

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We have obtained two classes of double lysogens that on induction yield higher titers of λ-d *dsd* transducing phage than of helper phage. One class was obtained by lysogenization of strain EM6116 (*dsd*$^{\Delta}$ *att*λ$^{\Delta}$ HfrC) with λ-*dsd* type 2 (*dsdC*$^{+}$ *dsdO*$^{+}$ *dsdA*$^{+}$, head-tail substitution). In the absence of either a normal *att*λ or the homology of a chromosomal *dsd* region, the transducing phage integrated at other sites, at least one of which, in strain EM6177, is near the origin of HfrC. On induction, strain EM6177 yields a phage burst of 20 to 50 with a λ*dsd*:λ ratio of 10^{4}:1. The asynchronously high yield of λ *dsd* is attributed to an efficiency of excision greater than that of λ. The other class was obtained by lysogenization of strain EM1407 (*dsdA att*λ^{+}) with λ-*dsd* type 2 (*dsdO6 dsdA*, partial deletion of *dsdC*). The DNA of mature λ-*dsd* type 2 is a complete dimer. It lacks nearly all of the phage late genes and b2 and carries about five bacterial genes. It could not be packaged as a monomer but is just within the packaging size limit as a dimer. Models for the derivation of these λ *dsd* phages and the high-yielding lysogens are presented.

We have previously described the isolation of two types of λ transducing phages carrying *dsd* (D-serine deaminase) genes (2). We sought such phages as a source of *dsd* deoxyribonucleic acid (DNA) to examine the regulation of D-serine deaminase (Dsdase) synthesis in vitro and to characterize the controlling elements involved in it. λ-*dsd* type 1, obtained by the generalized lysate method of Schrenk and Weisberg (13), carries the structural (*dsdA*$^{+}$), operator (*dsdO*$^{+}$), and regulatory (*dsdC*$^{+}$) Dsdase loci. λ-*dsd* type 2, obtained after the integration of λ into the *dsdC* gene by the method of Shimada et al. (15, 16), carries only *dsdA*$^{+}$, *dsdO*$^{+}$, and a fragment of *dsdC*$^{+}$. HFT lysates from lysogens for either of these phages contained preponderately wild-type λ, and wild-type and λ-d *dsd* particles were not separable on cesium chloride density gradients. We were unsuccessful in attempts to isolate plaque-forming λ-*dsd* phage. The phages were adequate for certain genetic experiments (2), but the λ-d *dsd* titers were too low to yield useful amounts of *dsd* DNA.

Lysates of a double lysogen containing both wild-type and defective (transducing) λ would be expected to contain equal numbers of each phage if excision and replication of the two phages were equally efficient. This is often the case; Press et al. (12) found equal quantities of φ80-d *arg* particles in lysates of several φ80-d *arg* lysogens. Defective phages lack one integrative end, however, and also may integrate into the homologous bacterial region rather than into the λ attachment site (*att* λ). Their excision may thus be more difficult than that of normal λ and so prejudice their yields, and indeed it seems to be fairly common to find that the yield of transducing phage in a lysate from a double lysogen is lower than that of the helper phage (12).

It seemed possible that conditions might be obtained such that the normal phage in a double lysogen would be at a replicative disadvantage relative to the transducing phage. In this paper we describe the properties of two such lysogenic systems, for types 1 and 2 λ-d *dsd* phage. On induction of appropriate double lysogens high-titer lysates of λ-d *dsd* are obtained, essentially free of helper phage.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial and phage strains utilized are described in Table 1.

Media and basic techniques. Preparation of LB plates, minimal medium plates with appropriate supplements for selection of transductants, and LB broth has been described previously (7). Tryptone broth, tris(hydroxymethyl)aminomethane-magnesium-gelatin (TMG) buffer, and EMBO plates were prepared as described by Shimada et al. (15). The liquid medium for bacterial cultivation was tryptone

TABLE 1. *Bacterial and phage strains*

Strain no.	Genetic constitution	Derivation
EM1106[a]	*dsdO1 dsdA1 pro*	EM1101 (2, 8)
EM1407[b]	*dsdC4 dsdA4 purF his try sup*$^+$ *att*λ$^+$	EM1003 (2)
EM1607	*dsdA6 purF his supF att*λ$^+$	(2)
EM3102	HfrH (*gal-uvrB*)$^\Delta$ (λ y199), Dsdase negative	HfrH (*gal-uvrB*)$^\Delta$
EM3126	*dsdA6 purF his supF att*λ$^+$ (λ y199 *dsdA*$^+$)	EM1607
EM3126-1	*dsdA4 purF his try sup*$^+$ *att*λ$^+$ (λ y199 *dsdA*$^+$)	EM1407
EM6116	HfrH *dsdC dsdA* (*gal-uvrB*)$^\Delta$	FB6001
EM6177	HfrH *dsdC dsdA* (*gal-uvrB*)$^\Delta$(λ *dsdC*$^+$ *dsdA*$^+$)	EM6116
FB6001	HfrH (*gal-uvrB*)$^\Delta$ (λ), Dsdase negative	(2)
FB6006[c]	HfrH (gal-uvrB)$^\Delta$ (λ), Dsdase constitutive	FB6001 (2)
FB7001	*dsdA6 purF his supF att*λ$^+$ (λ *dsdC*$^+$ *dsdA*$^+$)	EM1607 (2)
HfrH (*gal-uvrB*)$^\Delta$		M. Gottesman
MA140	*thr leu pro his thy*	W. K. Maas
MA140 (λ)[d]	*thr leu pro his thy* (λ)	MA140
λ	C_{1857} Sam 7	M. Gottesman
λ y199	C_{1857} Sam 7 *xis* 6 b515 b519	M. Gottesman
λ_{nin5}	C_{1857} *nin5*	(4)

[a] Strain EM1106 is a *pro* mutant of strain EM1101 (2, 8) obtained by penicillin selection (5) after *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis (1).

[b] Strain EM1407 is a *dsdC4 dsdA4* transductant of strain EM1003. P1 phage grown on strain EM1401 (*dsdC4 dsdA4 aroC*$^+$; reference 8) was used to transduce strain EM1003 to *aroC*$^+$. Because of close linkage some of the transductants also received *dsdC4 dsdA4*.

[c] FB6006 was selected as a derivative of FB6001 able to grow on minimal medium containing D-serine. It synthesizes Dsdase at a low constitutive rate and is presumably mutant either in a λ promoter or in *dsdO*.

[d] MA140 (λ) is a λ lysogen of strain MA140.

broth. The growth of P1 transducing phage and their use in transduction (7), assay of λ plaque-forming units (15), and the Dsdase assay (9) have been described. Tests for presence of cryptic λ genes in strain EM6116 were carried out by spotting about 10^7 each of λ phages harboring amber mutations in genes A, C, D, J, K, O and P on lawns of strain EM6116 on LB plates. The plates were then incubated at 39°C and observed for lysis. The phage were kindly provided by N. Glandsorff and M. Gottesman. Dsdase-negative lysogens, with λ y199 integrated into the *dsdC* gene, were isolated as described previously for λ C_{1857} Sam 7 (2).

Preparation of lysates from λ-*dsd* lysogens. The cells were cultivated to a density of 2×10^8 to 5×10^8/ml in tryptone broth, heat shocked at 41°C for 30 min, and then cultivated at 39°C for 2 to 4 h, all with shaking. The cultures were then centrifuged and suspended in TMG buffer. Chloroform was added (4%, vol/vol) and the cultures were shaken for 30 min at 37°C. The cell debris and chloroform were removed by centrifugation at 6,500 rpm for 15 min. Strain EM3126 lysed after one cycle of phage development because of the *supF* mutation. The lysates were treated with $CHCl_3$ and then subjected to low-speed centrifugation for clarification. The phage were concentrated by high-speed centrifugation as described below and resuspended in TMG buffer.

Transduction of *dsd* markers by λ-*dsd* phage. The method used was essentially that of Shimada et al. (15, 16). A 0.3-ml portion of a phage suspension in TMG buffer was mixed with 0.3 ml of bacteria (10^9/ml) that had been aerated for 1 h at 37°C in 0.01 M $MgSO_4 \cdot 7H_2O$, incubated for 30 min at 30°C, plated on appropriate selective plates, and incubated at 32°C for 48 to 72 h. When helper phage was used, it was added at a multiplicity of 5. For assay of *dsdA* transducing titers, strain EM1607 was always used as recipient.

Attempt to obtain λ-p *dsd*. In an attempt to obtain plaque-forming λ-*dsd*, phage lysates at various phage concentrations were plated with strain EM1607 as indicator on selective plates containing D-serine and incubated at 32°C. The edges of plaques that formed were examined for outgrowth of the background lawn of strain EM1607, which would indicate significant metabolism of D-serine by lysing cells infected with λ-p *dsd*. No such outgrowths were observed, and phage purified from plaques did not transduce *dsdA*$^+$ when tested with helper phage.

Bacterial matings. Strains were grown with slow shaking in LB broth to a density of about 10^9/ml at 30°C. A 5-ml portion of the recipient was then mixed with 0.8 ml of the donor and 5 ml of fresh LB broth. Growth of the mixture was continued with very slow shaking, and 0.1-ml samples were plated on selective media from time to time for scoring of recombinants.

Test for male-specific phage sensitivity. Stocks of male phages (R17 and M13) were prepared by a confluent plate lysis technique, and sensitivity to these phages was tested at 33°C as described before (11).

Purification of transducing phage particles. Lysates were spun at 10,000 rpm for 15 min, and the supernatants were centrifuged again at 40,000 rpm in a fixed-angle rotor 50 for 1 h at 4°C. The pellet was suspended in 10 ml of TMG buffer, and the low-speed and high-speed centrifugation was repeated once again. This time the pellet was suspended in 7.5 ml

of TMG buffer, and about 5.80 g of solid CsCl was added to adjust the initial refractive index to 1.38. Density gradient centrifugation was performed in a Spinco rotor 50 at 30,000 rpm for about 20 h at 6°C. Immediately at the end of the run the sample was collected in 2 drop-fractions by puncturing the bottom of the tubes.

After assays for plaque-forming and transducing particles (see above), the fractions that comprised the greater percentage of transducing particles were pooled together and recentrifuged in a CsCl gradient.

The resulting bands contained 0.1% of plaque-forming particles or less and were used for heteroduplex experiments. In all cases, the bands due to the helper and the transducing phages were visible and separate. The lower bands were much thicker and represented the transducing particles, whereas the upper bands were just visible and were helper phage. For λ-*dsd* type 1 in lysogen EM6177 the ratio of helper to transducing particles was about $1:10^4$; for λ-*dsd* type 2 in lysogen EM3126-1 the ratio was 1:10 to 2:10 as determined by ultraviolet absorption. The burst size with these two lysogens was 20 to 50.

Heteroduplex mapping. Heteroduplex formation and visualization of DNA under the electron microscope was performed according to Davis et al. (3) with the following modification.

A 1-μl sample of each λ-*dsd* phage (4×10^{11}/ml) in CsCl solution (as collected from the gradient) was mixed and added to 18 μl of 0.2 M disodium ethylenediaminetetraacetate, pH 8.4. The mixture was incubated at 4°C for 1 h and then diluted to a final volume of 50 μl with double-distilled water. Release of DNA from phages and concomitant denaturation was accomplished by the addition of 30 μl of 1 N NaOH followed by incubation at 25°C for 6 min. The solutions were neutralized by adding 30 μl of 1 M tris(hydroxymethyl)aminomethane, pH 8.4, and 200 μl of 0.2 M ethylenediaminetetraacetate (disodium salt), pH 8.4. The total volume (300 μl) thus obtained was dialyzed against 50 ml of renaturation buffer (14) for 1 h at room temperature. The renaturation step was followed by overnight dialysis at 4°C in a 0.1 M tris(hydroxymethyl)aminomethane-0.01 M ethylenediaminetetraacetate buffer, pH 8.4, containing 50% formamide.

We took λ y199 DNA as our standard of length, with an assigned value of 41.5 kilobases (kb) (this is based on measurements relative to λ_{nin5}, which gave a value for λ y199 DNA of 41.5 ± 0.4 kb [Palchaudhuri, unpublished data]). Other aspects of our electron microscope techniques and measurement by map measurer have been described elsewhere (10).

RESULTS

High-titer lysates of type 1 λ-*dsd*. When lysogenizing *Escherichia coli*, λ-d *dsd* and helper λ would be expected to integrate either into *att*λ or, by homology, into the *dsd* region. It seemed possible that in a strain lacking both, the phages might integrate into a site from which excision of λ-d *dsd* would be more competitive with that of λ than in previous lysogens. We therefore cured strain FB6006 (*att*λ^{Δ}, λ integrated into *dsdC*) by growing it at 39°C and screened survivors for ability to synthesize Dsdase. One survivor, denoted EM6116, which showed no detectable Dsdase synthesis and had thus apparently suffered deletion of *dsdA*$^+$, was selected for further work.

Strain EM6116 was transduced to *dsdC*$^+$ *dsdA*$^+$ with the previously isolated λ-d *dsd* type 1 (*dsdC*$^+$ *dsdA*$^+$; reference 2), using λ y199 as helper phage. The frequency of lysogenization without helper phage was less than 0.1% of that found with control strain EM1607; with helper phage it was about 0.1%. Twelve lysogens were purified and tested for ability to give rise to λ-*dsd* on heat induction. No plaque-forming λ-*dsd* were found. Seven of the lysogens gave low ratios of transducing to plaque-forming phage (<1:1,000) and were discarded. Five gave lysates with high ratios of transducing to plaque-forming units (Table 2). Lysogens 1 and 12 proved difficult to work with, as their lysates were very viscous due to large amounts of free DNA. Lysates of lysogen 4 were less viscous, and it was denoted EM6177 and used for further work.

The transducing:plaque-forming ratio in EM6177 lysates was usually about 5:1 with helper phage. In general, however, transduction titers underestimate actual number of transducing phage by 100- to 1,000-fold, even when helper phage are used (Palchaudhuri, unpublished data). Subsequent separation of transducing and plaque-forming phage in a CsCl gradient (see above) showed the actual ratio to be about 10^4:1. The phage did not transduce *aroC*, which is about 50% linked by P1 transduction to *dsdA* (7).

Because of the very low titer of plaque formers in its lysates, it seemed possible that

TABLE 2. *Phage stocks derived from λ-d dsd lysogens of strain EM6116*[a]

Lysogen no.	Plaque-forming titer (per ml)	Transducing titer[b] (per ml)
1	2×10^7	3×10^8
4	2.8×10^8	8.1×10^8
10	2.5×10^{10}	2.3×10^8
11	6.4×10^{10}	2.8×10^8
12	5.2×10^5	2.2×10^8

[a] A 800-ml portion of each lysogen was cultivated to a density of 5×10^8/ml at 30°C, shifted to 41°C for 30 min, and then shifted to 39°C for 3 h. The cells were then centrifuged, suspended in 30 ml of TMG buffer, and treated with $CHCl_3$ to induce lysis.

[b] Helper phage, λ y199, was added to the transducing mixture at a multiplicity of 5.

strain EM6177 might not harbor a wild-type helper phage, but that the replication of the defective λ-d *dsd* might be helped by cryptic late λ genes left behind when the parental strain was cured. We tested strain EM6116 for the ability to complement λ amber mutants for genes O, P, A, C, D, K, and J. No complementation was found. This strain thus does not carry a cryptic prophage, and the failure of the helper λ to appear in significant quantities in lysates is presumably due to poor excision from an abnormal *att*λ.

Location of λ in strain EM6177. Since strain EM6116 lacks *att*λ, and also at least part of the *dsd* region, the location of the prophage in the lysogen was in question. To locate it, we mated strain EM6177 and its original parent, strain EM6116, to the F⁻ strain EM1106 with selection for *pro*⁺, and to the F⁻ strain MA140(λ) with selection for *leu*⁺ or *pro*⁺. We did not select directly for *dsdA*⁺ as the marker is poorly expressed in this mating, but scored *pro*⁺ recombinants for *dsdA*⁺ by replica plating. We do not know which *pro* gene is mutant in strain EM1106, but have previously found it to be closely linked to *lac* by interrupted mating (unpublished data). The results are presented in Table 3. Because of very low transfer from strain EM6177, the matings were not interrupted. It may be seen that the numbers of recombinants were much lower with the lysogen EM6177 as donor than with its parent. This does not seem to be due to a chance mutation of the F factor, as similarly reduced transfer was observed when two other λ-*dsd* lysogens of strain EM6116 were used as donors (data not shown). Such transfer of *dsdA*⁺ to the recipient strain EM1106 as occurred was very closely linked to that of *pro*⁺, a proximal marker for this type of donor (HfrH), as 81 of 100 *pro*⁺ recombinants tested proved to be *dsdA*⁺. All these donor strains (EM6177, EM6116, etc.) were equally sensitive to male-specific phages (R17 and M13), indicating the presence of normal F-pili. The transfer genes affecting the growth of pili, which are transferred last during bacterial conjugation, were therefore apparently not affected by the integration of λ. These results suggest that the λ-d *dsd* may have integrated into the early part of the F factor interfering with one of its transfer functions (6).

High-titer lysates of λ-*dsd* type 2. We had previously isolated type 2 λ-d *dsd* by the method of Shimada et al. (2, 16). λ lysogens of strain HfrH (*gal-uvrB*)$^{\Delta}$ with λ C_{1857} Sam 7 integrated into *dsdC* were obtained by penicillin selection (5) and on heat shock gave LFT lysates of λ-d *dsd* phage (2). We were not able to obtain plaque-forming λ-*dsd* phage or to obtain secondary lysogens yielding a satisfactory ratio of transducing to plaque-forming particles. We therefore repeated the isolation procedure using λ y199, which has two extensive deletions in the λ b2 region. We hoped thereby to obtain plaque-forming λ-*dsd*, as λ y199 can carry more bacterial DNA than λ of normal density.

TABLE 3. *Transfer of leu⁺ and pro⁺ by strains EM6116 and EM6177*

Cross	Time of plating (min)	*leu*⁺	*pro*⁺
EM6116 × MA140 (λ)	0	200	200
	30	500	300
	60	ND[a]	ND
	180	ND	ND
EM6177 × MA140 (λ)	0	0	1
	30	1	5
	60	3	7
	180	11	35
EM6177 × EM1106	0		3
	30		13
	60		27
	120		93

[a] ND, Not done.

Ten λ y199 Dsdase-negative lysogens were isolated from five separate cultures. The negative phenotype could have arisen from integration of λ either into *dsdC* or *dsdA*. In all cases a low but detectable level of Dsdase (specific activity, 0.5 to 0.8) was formed constitutively by the cells. Dsdase synthesis was not further inducible. This synthesis, insufficient to allow growth in the presence of DS, had been noted with previous λ integration lysogens and attributed to read-through from the λ *int* promotor (2, 16). Of 40 such lysogens obtained with normal density λ in several previous experiments, all showed this phenotype. It suggested that the phage had integrated into *dsdC* with *int* adjacent to *dsdO* and that the lysogens on induction would give rise to λ-d *gal* type transducing phage. Thus *dsdC*, like certain loci described by Shimada et al. (16), would appear to contain at least one preferred site for λ integration, with a preferred orientation for that integration: *int* adjacent to *dsdO*.

LFT lysates were obtained from several lysogens, and one, from a lysogen denoted EM3102, was used to transduce strain EM1607 (*att*λ⁺, *dsdA* point mutant) to *dsdA*⁺, with wild-type λC_{1857} Sam 7 as helper. The lysogens were induced to give HFT lysates by heat shock. Of 50 lysogens, all but one yielded low ratios of transducing to plaque-forming phage, less than $1:10^4$. The one high yielder, denoted EM3126,

showed a ratio of 1:100 with helper phage and a plaque-forming titer of 2.1×10^7/ml (burst size, 0.05 to 0.1). We attempted to isolate plaque-forming λ-*dsd* from this strain without success.

Strain EM3126 has two disadvantages as a lysogen. It harbors *supF*, which suppresses the amber mutation in the phage lysis function. Harvesting of phage is therefore difficult, and the yield is poor. Moreover, its lysates were so viscous that it was extremely difficult to concentrate and purify the phage. We therefore transduced strain EM1407 (*dsdC dsdA4 supF*$^+$ *att*λ$^+$) to *dsdA*$^+$ with a lysate from strain EM3126, again using normal-density λC_{1857} Sam 7 as helper. Four transductants were purified and subjected to heat shock; they released large numbers of λ-d *dsd* particles. One, which yielded a lysate with a transducing to plaque-forming ratio of 5:1 to 10:1 (see above) and a plaque-forming titer of 4.8×10^9/ml, was denoted EM3126-1 and used in further work.

Dsdase synthesis in λ-*dsd* type 2 lysogens. Dsdase synthesis in strain EM3126 is constitutive at two- to threefold the low rate observed previously in strain EM3102 and is hyperinducible. This was to be expected, as the genetic constitution of strain EM3126 is *dsdC*$^+$ *dsdO6 dsdA*/λ*dsdC*$^-$ *dsdO*$^+$ *dsdA*$^+$. Strain EM3126-1, however, proved to be a very high constitutive, with a Dsdase specific activity of 40 during the logarithmic phase of growth. Two of the other transductants were also high constitutives; the third behaved analogously to EM3126. We thought at first that the constitutive activity in strain EM3126-1 might be due to the presence of the *dsdC*^{c}*4* allele on the EM3126-1 chromosome. Constitutive synthesis in *dsdC*^{c}*4* strains is, however, low and subject to catabolite repression (8); synthesis in strain EM3126-1 was not subject to catabolite repression. A specific activity of 40 is very high—twice what we observe in *dsdO6* strains. Moreover, when we used λ-*dsd* type 2 harvested from strain EM3126-1 to transduce strain EM1607 (*dsdC*$^+$ *dsdO6 dsdA*$^-$) to *dsdA*$^+$, the transductants also formed Dsdase at a high constitutive rate. We conclude that in the excision of λ-*dsd* type 1 from strain EM3126 exchanges sometimes occurred between the chromosomal and phage *dsd* regions (see below) to yield *dsdO6 dsdA*$^+$ recombinant phages.

Heteroduplex mapping of λd *dsdC*$^+$ *dsdA*$^+$ (λ-*dsd* type 1). λ-*dsd* type 1 was derived from a structurally normal λ (no deletions). To determine whether the transducing particles were substituted in the right or the left arm, we hybridized its DNA with that of λ y199, which has suffered deletions in the b2 region (Fig. 1a). Figure 1b shows the heteroduplex between λ*dsd* type 1 and λ y199. Bacterial DNA with a length of 20.7 kb has replaced the head-tail genes (from A through *att*). This conclusion is based on the fact that although the transducing phage was constructed from wild-type lambda phage, it does not show any b2 deletion loops when hybridized with λ y199 (Fig. 2). Helper phage that was obtained as the lighter band during phage purification shows the two b2 deletion loops (Fig. 1a) when hybridized with λ_{nin5} (nin deletion) and is therefore confirmed to be normal λ y199. The large substitution loop of the λ-*dsd* type 1/λ y199 heteroduplex molecule starts from 5.5 kb (measured from the left end) and extends to about *att* (Fig. 1b). The location of *att* (20.5 kb from the right end) is assigned by measuring the length of the double-stranded part of the heteroduplex molecule. The longer arm of the substitution loop representing the bacterial DNA provides enough space to accommodate the *dsdA*$^+$ gene, its regulatory genes (*dsdO*$^+$ *dsdC*$^+$), and also a portion of the neighboring bacterial chromosomal region, 15–16 genes.

Heteroduplex mapping of λ-*dsd* type 2. λ-*dsd* type 2 was derived from λ y199. To determine its structure, we hybridized it to λ_{nin5}. The heteroduplex molecule (Fig. 1c and 3) shows a big substitution loop, a small deletion (or insertion) type loop, and a long single-stranded region. The region carrying the two b2 deletions is not seen and so is missing from λ-*dsd* type 2. The small deletion loop (length 2.3 kb) corresponding to the nin5 deletion of λ_{nin5} has been used as a reference loop. The location of this well-characterized nin5 loop is 13.0 kb away from the *att* site (4). The long arm (length, about 25.5 kb) of the substitution loop must be the phage DNA of λ_{nin5}, and the short arm (4.5 kb) is chromosomal DNA of the transducing phage. This means that almost all of the phage DNA (25.5 kb) at the left side of the *att* site has been replaced by a much shorter piece of chromosomal DNA (4.5 kb). The chromosomal DNA must be carrying the *dsdA*$^+$, *dsdO*$^+$, and part of *dsdC* (see genetic data) loci and may not have much space left for other markers. Interestingly enough, the long unpaired single-stranded region of the heteroduplex molecule has the same length (about 26 kb) as is obtained by adding up all of the different parts of the λ-*dsd* type 2 DNA that hybridized with λ_{nin5}. It is also clear that the length of the hybridized part of the phage λ-*dsd* type 2 falls extremely short of the normal λ genome.

In the same experiment, we found two other different heteroduplex structures (Fig. 1d and

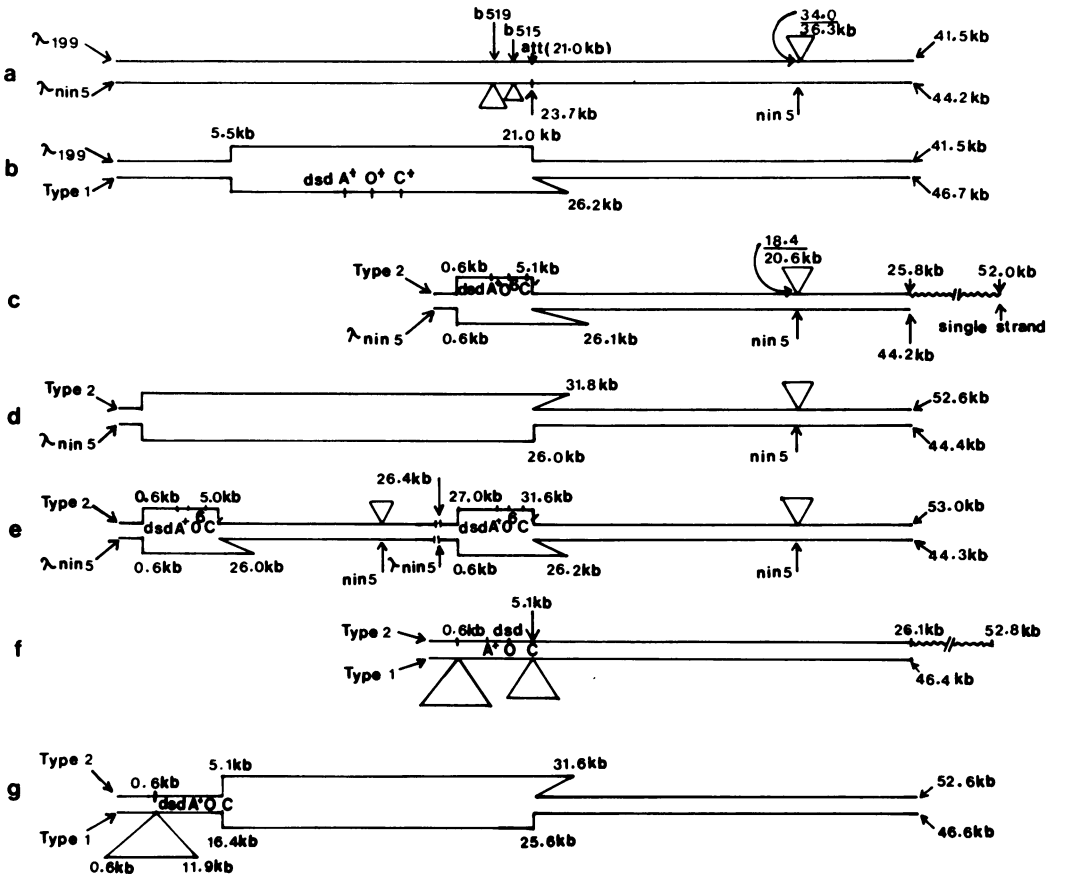

FIG. 1. *Schematic diagrams of heteroduplexes between λ-dsd types 1 and 2 and reference λ phages. The (b), (c), (e), (f), and (g) heteroduplexes are shown in Fig. 2, 3, 4, 5, and 6, respectively. Coordinates are in kilobases (kb).*

e). In the second structure (Fig. 1d) the long single-stranded region of Fig. 1c has disappeared but the nin5 loop is seen at the right position (about 13 kb away from the *att* site and 6 kb away from the right end). Further, the disappearance of the single-stranded region has resulted in an equal increase in the short arm from 4.5 kb to 31.6 kb. The other arm (length, about 25.5 kb), which remained unaltered, must be the phage DNA of λ_{nin5}. The results indicate that the intrinsic transducing phage, λ-*dsd* type 2, is quite small in size but may be duplicated in tandem to give a total length of about 53 kb.

These two heteroduplex structures (Fig. 1c and d) can be explained in the following way if one accepts that the λ-*dsd* type 2 phage is a duplication. The first structure (Fig. 1c) is obtained if the annealing starts at the head-tail ends and continues sequentially. The second structure (Fig. 1d) results when the annealing starts from both ends of the partner molecules simultaneously.

The best evidence for the duplication comes from the third structure (Fig. 1e and 4), where two λ_{nin5} molecules have hybridized with one molecule of λ-*dsd* type 2. Two identical substitution loops are observed at equal intervals with reference to nin5 loops. The long arm (length, 25.5 kb) of the substitution loops must come from λ_{nin5}. These substitution loops and their assigned positions with respect to the nin5 loops are very similar to the first structure shown in Fig. 1c. Measurements indicate that the segments of λ-*dsd* type 2 DNA, which separately anneal with λ_{nin5} DNA molecules, are exactly the same length and have exactly the same structure. Helper phage were similarly characterized and confirmed to be wild-type λ.

Self-hybridization. The DNAs of the two purified transducing phages were also extracted separately, and their lengths were de-

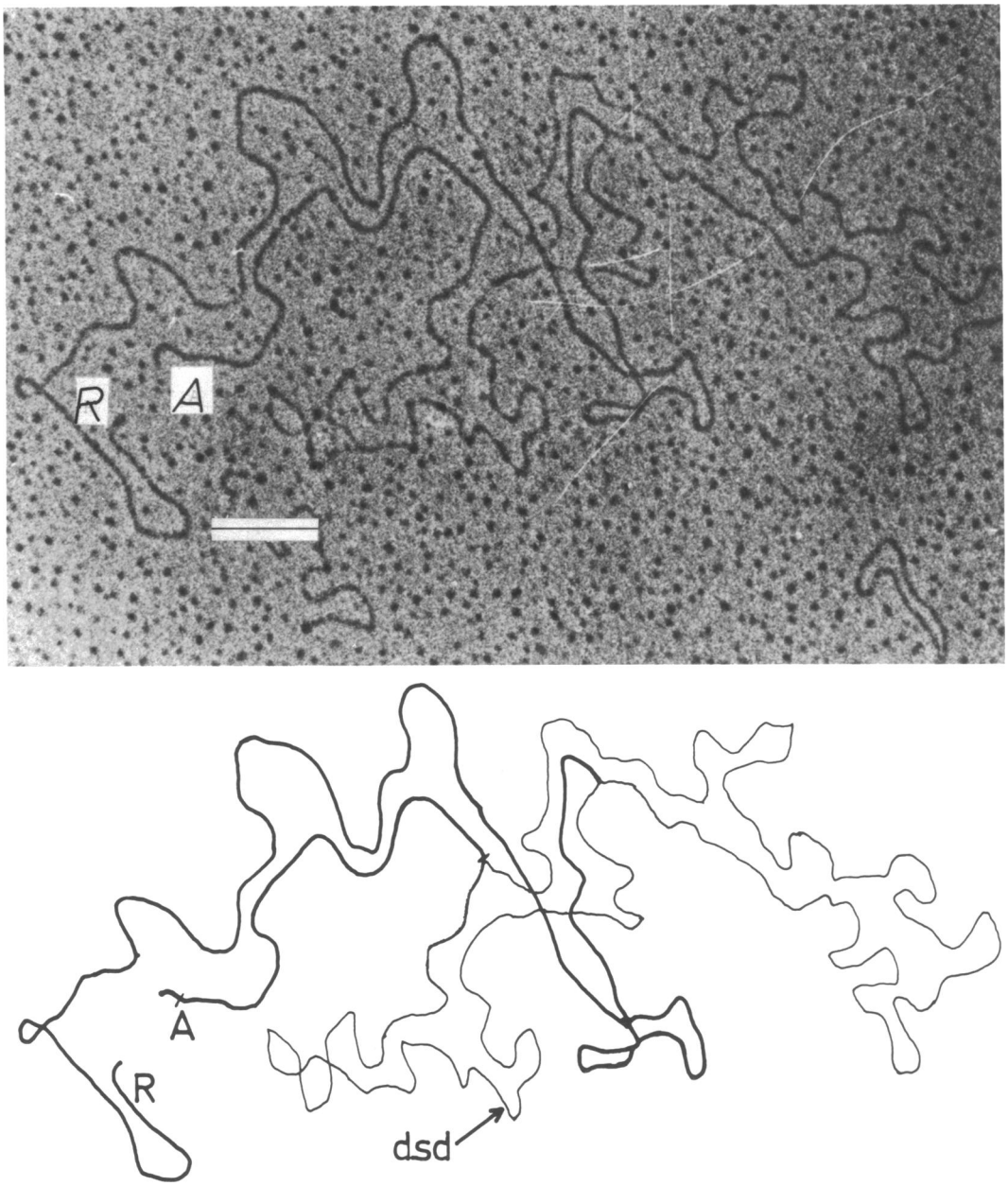

FIG. 2. *Electron micrograph of heteroduplex between λ-dsd type 1 and λ_{p199} DNAs. Bar represents 1 kb. The structure of this molecule is depicted in Fig. 1b. A tracing of the heteroduplex molecule is also shown; double-stranded DNA is represented as a heavy line, and single stranded DNA is represented as a light line.*

termined. λ-*dsd* type 1 is comparable with wild-type λ (about 46.8 ± 0.2 kb). λ-*dsd* type 2 is 53 ± 1 kb in length. These DNAs were denatured, and the resulting single strands were examined under the electron microscope to test for heterogeneity in the population. Most of the single-stranded DNAs were found to have the same lengths as their double-stranded forms, i.e., 46.6 ± 0.8 kb for λ-*dsd* type 1 and 53.4 ± 1 kb for λ-*dsd* type 2.

Heteroduplex mapping between λ-*dsd* type 1 and λ-*dsd* type 2. To determine the extent and the orientation of the chromosomal genes present in the two transducing phages and their

integration and excision sites, they were mixed in equal amounts and denatured and renatured as described in Materials and Methods. Two different structures (Fig. 1f and g) are seen in the same experiment, depending on which region of the duplicated phage DNA interacts with the DNA of the λ-*dsd* type 1. If the reannealing begins from the head-tail ends of the phage genomes and progresses sequentially, the resulting structure is that shown in Fig. 1f and 5. In addition to the bacterial segment shared by λ-*dsd* type 1 and λ-*dsd* type 2, λ-*dsd* type 1 carries two additional segments on both sides of a homologous region, which are reflected as two insertion loops. The first insertion loop, of size 11.3 kb, is located at about 0.6 kb from the left end, and the second insertion loop, of size about 8.9 kb, is located at the position of the *att* site. In addition, a single-stranded unpaired DNA of length 27 ± 1 kb is present at the right end and looks comparable to the single-stranded region of the heteroduplex λ-*dsd* type $1/\lambda_{nin5}$ molecule (Fig. 1c). The duplicated phage, λ-*dsd* type 2, has about 0.6 kb of the head genes at one end, and this accounts for the first double-stranded part in the hybrid. Thus, the first part of the insertion loop is due to 4.9 kb of the unpaired head-tail genes of the type 1 phage (5.5 kb − 0.6 kb), and the rest is the bacterial genes (11.3 kb − 4.9 kb = 6.4 kb) present between *dsdA* and *aroC*. The second insertion loop, of length about 9 kb, represents bacterial genes between *dsdC* and *supN* carried by phage *dsd* type 1.

Figure 1g provides further confirmation to the above conclusion; the only difference with Fig. 1f is that the right side insertion loop forms one arm of a big substitution loop. The other arm of this substitution loop is about 26.5 kb, which is almost the total length of λ-*dsd* type 2 before duplication. The corresponding micrograph is shown in Fig. 6. This structure is derived when the reannealing takes place from both ends of the phage genomes simultaneously. Our findings confirm that the λ-*dsd* type 2 phage has the same orientation of bacterial genes as λ-*dsd* type 1. They also provide independent confirmation of the A - - - - *dsdA dsdO dsdC* - - - - *int* gene orientation shared by the two phages. In the lysogens the phages were inserted at two different sites but on the same side of $dsdC^+$ (counterclockwise to *dsdC*), and the excision sites were also different.

To trace the origin of this duplication we also did heteroduplex analysis of the transducing phage from strain EM3126, which was used to form strain EM3126-1. Heteroduplex mapping indicated that this precursor phage is also duplicated and structurally identical with λ-*dsd* type 2.

DISCUSSION

Our λ-*dsd* type 1 phage ($dsdC^+$ $dsdA^+$) appears to have been formed by a classical λd *gal* excision event, with late genes replaced by bacterial DNA. The length of its DNA is about the same (46.8 kb) as normal λ (46.5 kb), so it incorporated about as much bacterial DNA as it lost phage DNA. A model that is consistent with its formation is presented in Fig. 7. We propose that on lysogenizing a normal host with a normal *att*λ and a normal *dsd* region except for a point mutation in *dsdA* as in our previous experiments (2), the λ-*dsd* type 1 phage and its wild-type helper integrated at one of these two sites, probably *att*λ. On prophage activation the λ-*dsd* was at a replicative disadvantage to the helper because of difficulty in excision, hence the low yield that we reported. In the Δ (*att*λ *dsd*) host EM6116, the λ and *dsd* recognition sites were missing. The difficulty in forming lysogens with such a host was reflected in the low efficiency of its transduction to $dsdA^+$ $dsdC^+$ by λ-*dsd* type 1. Once lysogens such as EM6177 were formed, however, the λ-*dsd* phage often had a considerable advantage in replication over the helper (Table 2).

We have no real evidence as to whether the sites of attachment for the two phages are the same in the various Δ (*att*λ *dsd*) lysogens. The λ-*dsd* integration site, at least in strain EM6177, appears to be very near to the origin of HfrC and perhaps in the F factor. The data of Table 2 do suggest, however, that the sites for λ and λ-*dsd* may be the same, with the order of the phages accounting for the difference in yield of plaque formers (Fig. 8). Both λ-*dsd* and helper phage would probably have difficulty in excising by the site-specific *int-xis* mechanism from an abnormal *att* (B* B'*), and significant excision by homology (general recombination) or possibly *ter* cutting in the *cos* regions could be expected from a tandem lysogen (18). Such excision from a tandem lysogen with the structure shown in Fig. 8a would yield wild-type λ, whereas a structure as shown in Fig. 8b would yield λ-*dsd*. If *int-xis*-mediated site-specific excision of the helper phage from the tandem lysogen in Fig. 8b were extremely rare, the excision product would then be almost entirely λ-*dsd*, as we observed with lysogens 1, 4, and 12 (Table 2). Alternatively, as all the λ-*dsd* type 1 lysogens released about the same sized burst of λ-*dsd*, the λ-*dsd* phage may have had a preferred secondary *att*, whereas the helper phage

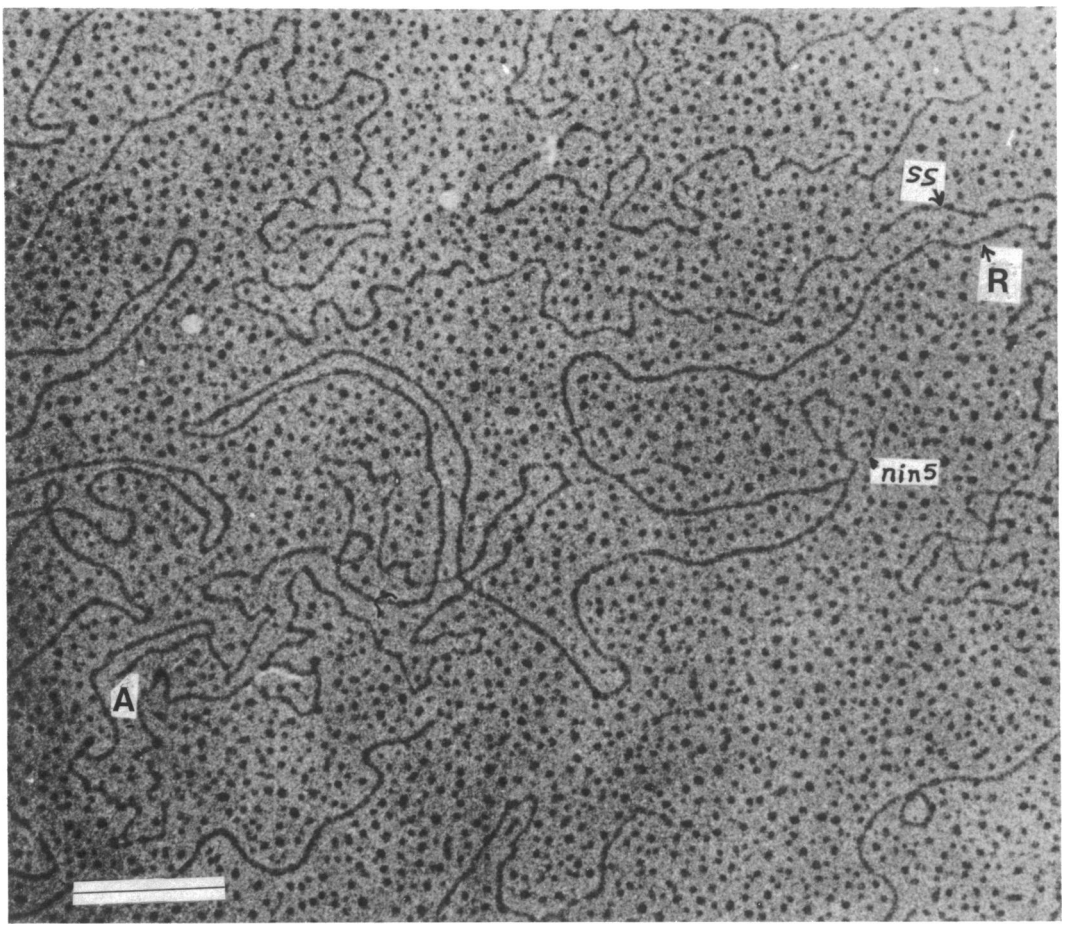

FIG. 3. *Electron micrograph of heteroduplex between λ-dsd type 2 and λ_{nin5} DNAs. Bar represents 1 kb. The structure of this molecule is depicted in Fig. 1c. A tracing of the heteroduplex molecule is also shown; double-stranded DNA is represented as a heavy line, and single-stranded DNA is represented as a light line. Some of the features are labeled in the micrograph to correspond with the labels in the tracing.*

integrated at a number of secondary sites from which it subsequently excised with varying efficiency. In spite of the inability of the resident helper phages to replicate efficiently in strain EM6177—only 1 in 200 to 500 lysogens yields a plaque former—their late genes are sufficiently expressed to provide tail structures for the 20 to 50 λ-*dsd* particles that emerge from the average burst.

λ-*dsd* type 1 carries about 6.4 kb of the *E. coli* chromosomal region between *dsdA* and *aroC* and about 9 kb of the region between *dsdC* and *supN*. We tested it for ability to transfer *aroC*$^+$, with negative results. It would not be expected to do so, as *aroC* is only about 50% linked to *dsdA* by P1 transduction (7), which corresponds to a distance of 15 to 20 kb (Palchaudhuri, unpublished data). We have not tested for transfer of *supN* but would also not expect it on the basis of P1 cotransduction frequency (23%; reference 7).

The formation of λ-*dsd* type 2 (*dsdA*$^+$) is more difficult to explain. This phage has lost DNA from its *attP* site almost to the cohesive end site. It has incorporated 4.5 kb of bacterial DNA—about 5 genes—including *dsdO*, *dsdA*, and part of *dsdC*. It is, within the limits of our measurements of the heteroduplex molecules, a complete dimer. During its excision from strain EM3126 it incorporated the *dsdO6* allele from the bacterial genome in place of the *dsdO*$^+$ allele and carries it in both of its *dsd* segments. Its frequency in lysates from both strains EM3126 and EM3126-1 is significantly greater (5 to 10×) than that of the helper phage, although both strains contain a normal *att*λ.

A possible model for formation of λ-*dsd* type 2 is presented in Fig. 9. We assume that the

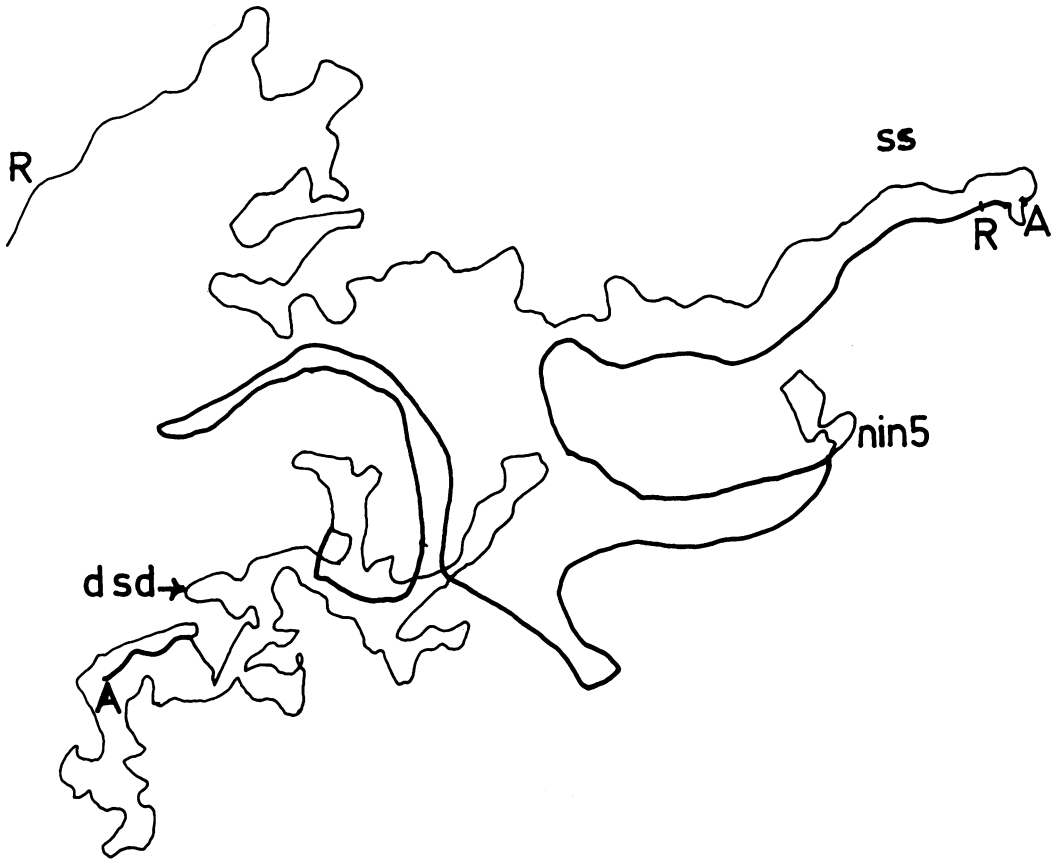

FIG. 3

original phage DNA, produced by faulty excision from strain EM3102, was the monomer. Such a DNA, slightly more than half the size of a normal λ DNA molecule, could not be packaged (17). It should replicate, however, generating linear concatemers. Cuts at every other cohesive end site would yield dimers, which are just within the limit amount of DNA that can be packaged (17). The matured dimers are then the λ-*dsd* type 2 phage. The presence of two cohesive ends per packagable DNA may explain the rather high viscosity of our lysates; monomer cuts could occur and would yield DNA that could not be matured and so would remain in solution.

On infecting strain EM1607 (*att*$^+$) with helper phage to form the lysogen EM3126, we assume that the dimers integrated by homology into the *dsd* region. Integration by homology had to be between the *dsdO6* mutation and the left end of that portion of *dsdC* homologous to the fragment of *dsdC* that is carried on the phage to explain the pickup of the *dsdO6* mutation on excision. The helper could have integrated normally at *att*λ, or by homology into one of the defective monomers as we suggest in Fig. 9. On induction the defective prophage would excise as the monomer and replicate. A monomer excising by homology from the *dsd* region need not excise exactly as it integrated. To be recognized by our selection procedure it must retain *dsdA*$^+$, but this still allows it to excise to the right of the original integration points, up to the chromosomal *dsdA* point mutation. Thus, such an "incorrect" excision of the monomer adjacent to *dsdO6* could allow it to pick up *dsdO6* in place of *dsdO*$^+$ (Fig. 9). Replication and maturation of such a DNA would then yield mature phage whose DNA is identical to that of its predecessor except that *dsdO6* replaces *dsdO*$^+$. Such an exchange had occurred with three of the four λ-*dsd* type 2 phage we examined that were derived from strain EM3126.

This model is fairly simple and seems to fit all of our data. Because of the low burst size

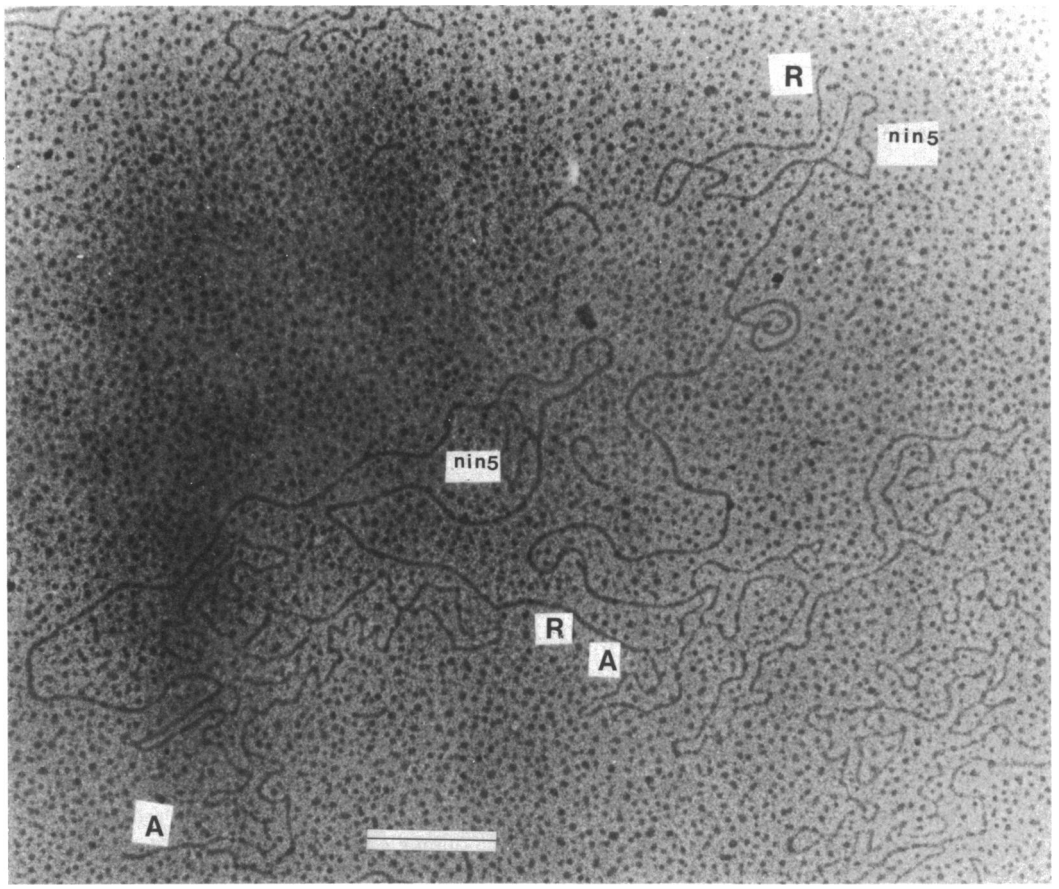

FIG. 4. *Electron micrograph of heteroduplex between one λ-dsd type 1 and two λ_{nin5} DNAs. Bar represents 1 kb. The structure of this molecule is depicted in Fig. 1e. Some of the features are labeled in the micrograph to correspond with labels in the accompanying tracing.*

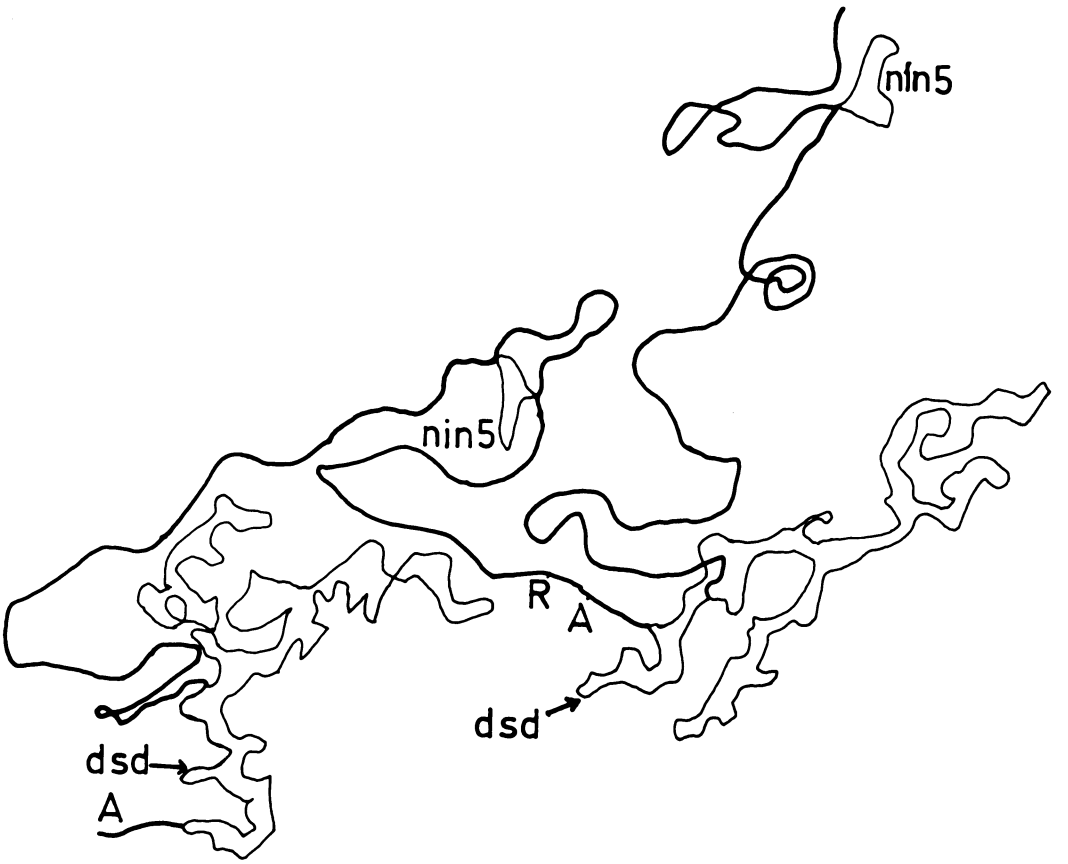

FIG. 4

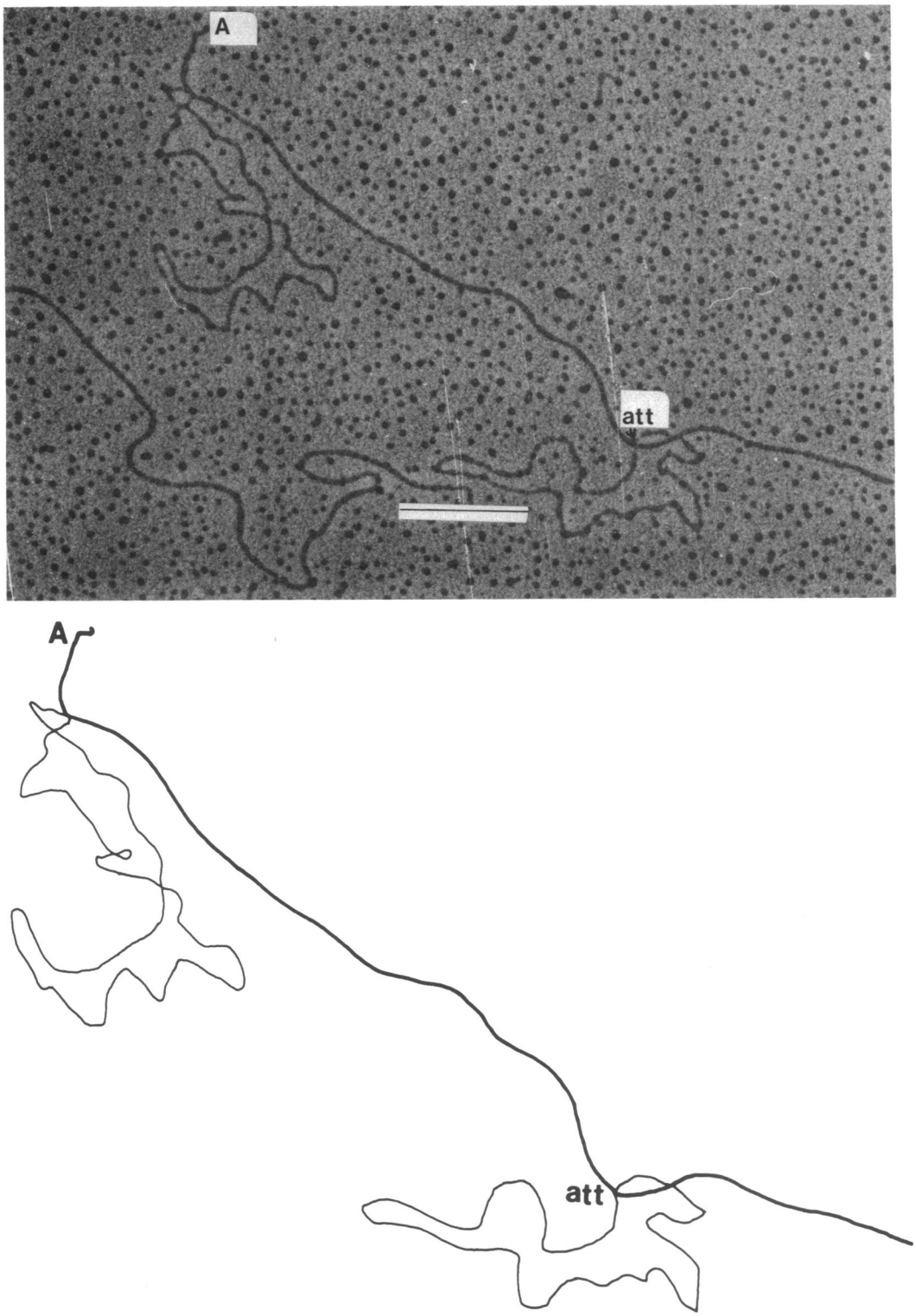

FIG. 5. *Electron micrograph of heteroduplex between λ-dsd type 1 and λ-dsd type 2 DNAs. Bar represents 1 kb. The complete structure is depicted in Fig. 1f. The micrograph shows a broken molecule. The region of the heteroduplex to the right of the insertion loop at att is incomplete in the micrograph. A tracing is also presented.*

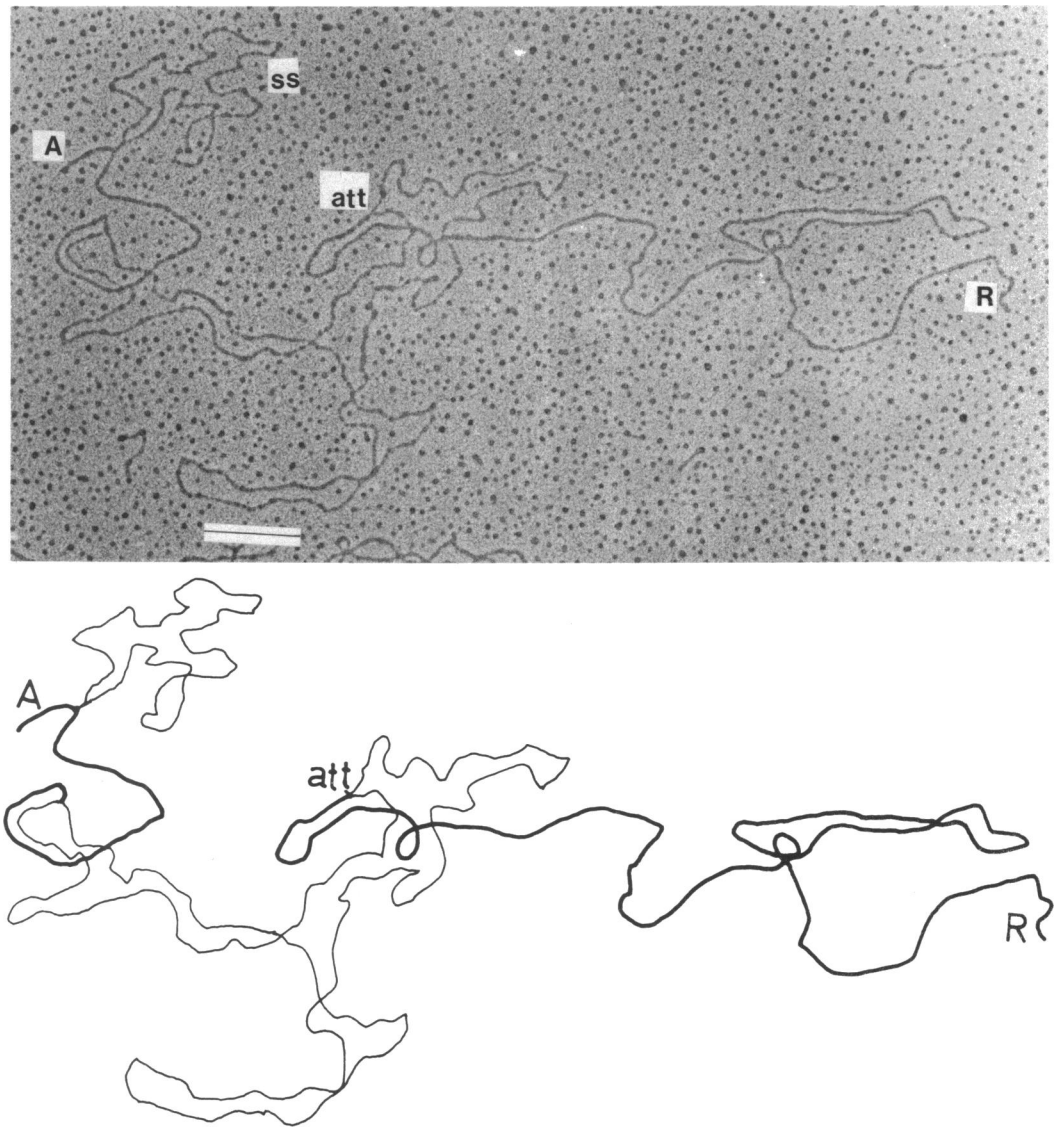

FIG. 6. *Electron micrograph of heteroduplex between λ-dsd type 1 and λ-dsd type 2 DNAs. Bar represents 1 kb. The same structure is depicted in Fig. 1g. A tracing is also presented. Heavy lines are duplex; light lines are single strand.*

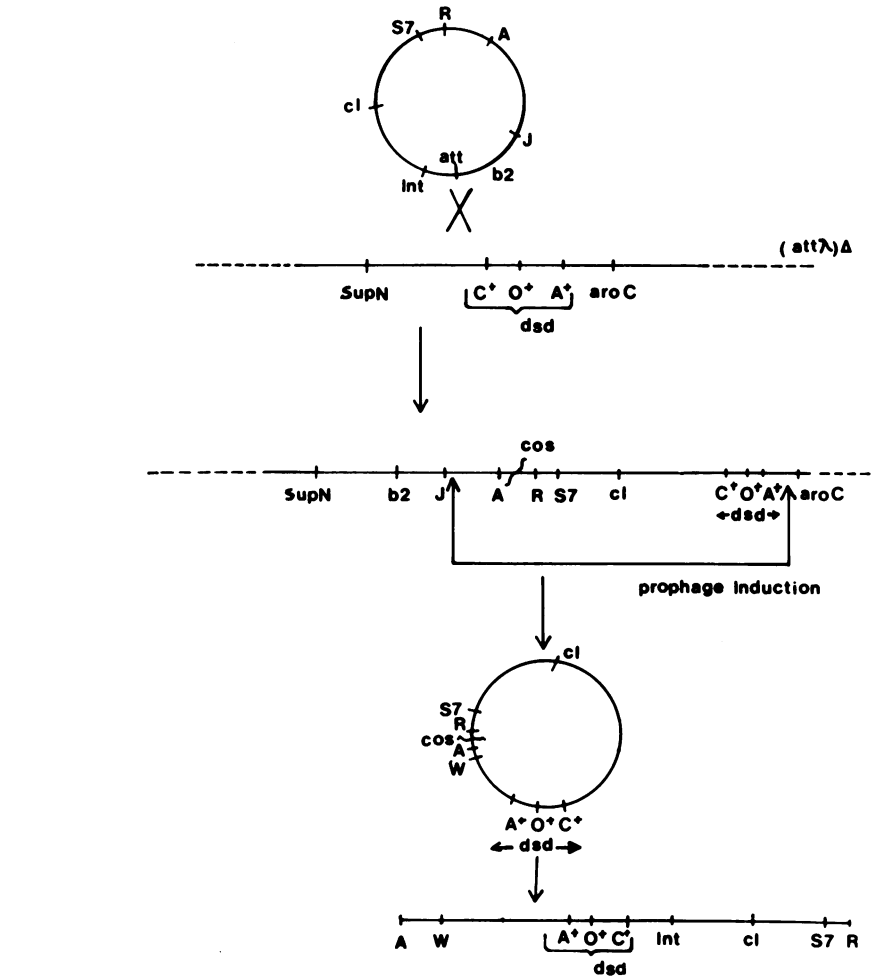

FIG. 7. *Sequence of events involved in the formation of λ-dsd transducing phage type 1. Details of each step are explained in the text.*

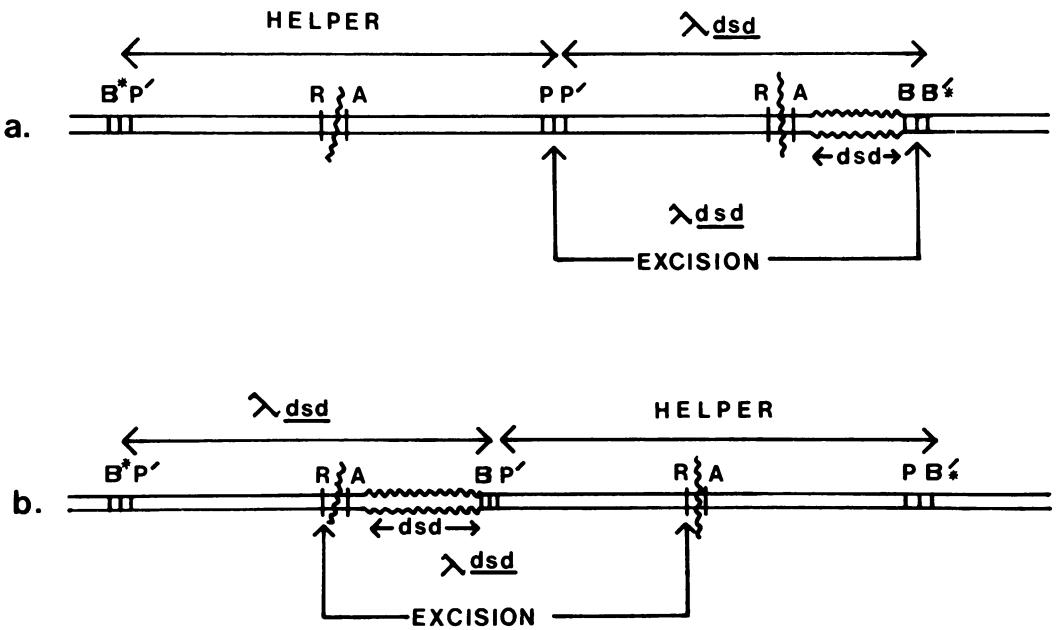

FIG. 8. *Excision of λ-dsd type 1 from tandem lysogens.*

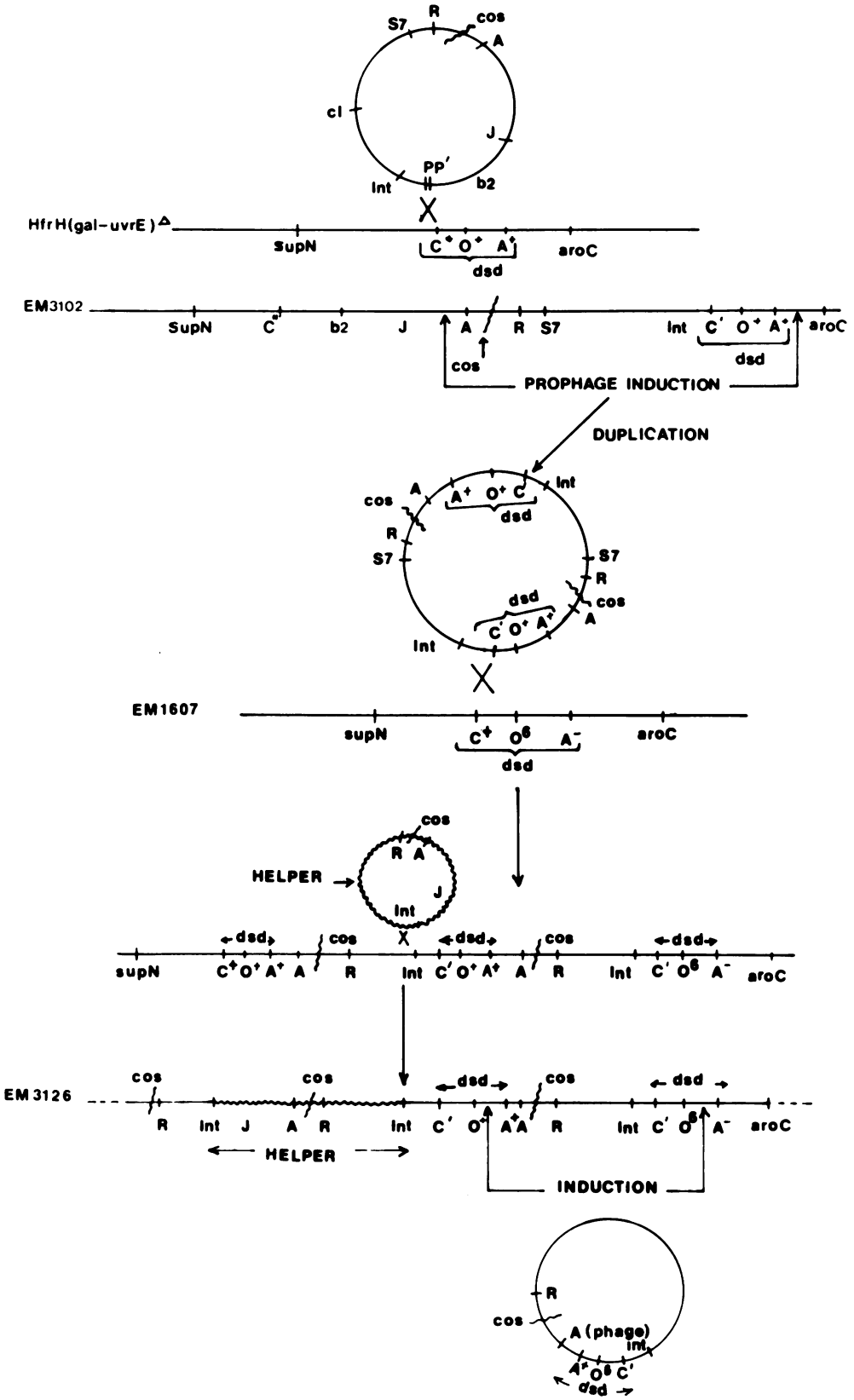

FIG. 9. *Sequence of events leading to the formation of λ-dsd transducing phage type 2. Details are explained in the text. The right and left portions of the dsdC gene, which is split by integration of λ, are designated C' and C'', respectively.*

(0.05 to 0.1) with strain EM3126, it is likely that the helper phage in that strain was not integrated at *att*λ but at the *dsd* region; the higher burst (20 to 50) with strain EM3126-1 suggests integration of both phages at *att*λ in that strain. With two monomers per helper, the transducing phage would have a twofold replicative advantage over the helper phage. If one assumes a slightly higher efficiency of excision for the λ-*dsd*, the latter's numerical superiority over the helper in the burst is also readily explained. Normal excision would be expected from *att*λ, and the λ-*dsd* would therefore retain *dsdO6* as we observed it to do.

Formation of a lysogen such as EM3126 is not a common event—it was 1 out of the 37 lysogens that we examined which were formed by transducing phages from strain EM3102—but the screening procedure that led to its isolation was not unduly tedious. It has given rise to a λ-*dsd* that should be useful for development of a Dsdase in vitro system, as the *dsdO6* mutation obviates the need for regulator gene product, inducer, and cyclic adenosine 5′-triphosphate system (2). The phage may also be useful for obtaining λ-*dsd* type 2 phage carrying other *dsdO* mutations.

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