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Evidence for the Site of Lambda Insertion in the *ilv* Gene Cluster of *Escherichia coli* K-12

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The experiments reported herein provide evidence that the secondary site of λ is in the *ilvC* instead of the *ilvA* gene.

Bacteriophage λ can integrate into *Escherichia coli* by recombination between the prophage and bacterial chromosomes at specific attachment sites (*att*'s) in each DNA molecule (2). There is one *att* on the phage chromosome (*att*P.P') and another on the bacterial chromosome (*att*B.B'). The maximal integration frequency does not occur if either *att* is missing or altered (1, 3). The integration process also requires a protein (Int) which is encoded by the *int* gene of the phage (6, 15) and which specifically promotes recombination at the attachment sites (5, 14). It has been further shown that λ can insert into sites other than *att*B.B' providing that this site is deleted in a recipient strain and the integration frequency of bacteriophage λ in such a mutant strain was reduced about 200-fold relative to integration into wild-type *E. coli* (12). This residual integration, like normal integration, requires the *int* gene product, and λ occurs by a crossover at the normal attachment site on the prophage chromosome (12). Analysis of the resulting lysogens has indicated that certain sites on the *att*B.B'-deleted chromosome are frequently used for λ insertion and that lysogens in which λ has inserted into or near a specific gene, thereby inactivating its function, can be obtained by the appropriate selection technique (12).

Shimada et al. (13) have reported that λ induces *pro* mutations far more frequently than any other kind. Other mutations that they found to be induced by λ were *nad, gua, trp, ton, mal, leu, txs, gal,* and *ilv*. Of the *ilv*-induced mutations, Shimada and co-workers concluded that the site of λ insertion in the *ilv* gene cluster was in the *ilvA* gene. This study was undertaken to examine further the site of the insertion of λ in the *ilv* cluster since there is discrepancy in the literature on the specific site.

In this paper, we report evidence that the secondary site of λ insertion is in the *ilvC* instead of the *ilvA* gene as previously described (13). The bacterial strains and phages used in this study are listed in Table 1. To obtain λ insertion strains, *E. coli* strain KS302 [HfrH Δ(*gal-att*λ-*bio-uvrB*) *thi*] was employed, and the experimental procedures were as described by Miller (9). After about 48 h, the small colonies were picked from plates and tested for Ilv$^-$, the λ lysogen phenotype. For λ transductions, lysates were prepared by thermal induction of lysogens, using the procedure described elsewhere (9). Transduction techniques were those employed by Shimada et al. (13). To obtain phage carrying bacterial DNA from the right side of prophage, induced lysogens were used to select IlvA$^+$ transductants. Transducing phage lines carrying bacterial markers from the left (*cya*) side of the insertion were selected directly by transduction of the appropriate *ilvC* and *cya* mutants.

Shimada et al. (12, 13) have described isoleucine and valine auxotrophs arising from rare integration events in which the phage λ was integrated into the *ilv* region. On the basis of mutants supplied to these workers, they concluded that the integration had occurred in the *ilvA* region. Actually, their data showed that λ was inserted between a marker designated *ilvA206* and several other *ilvA* markers. As the data in Table 2 show, a strain carrying the *ilv-206* marker is quite clearly an *ilvC* mutant since an extract prepared from it had threonine deaminase activity but lacked isomeroreductase activity. Furthermore, extracts prepared from strain KS505 and several apparently comparable *ilv* strains prepared by insertion of λ into the *ilv* region in this laboratory (strains CU431 and UK51) exhibited activities for all the isoleucine and valine biosynthetic enzymes except the isomeroreductase (Table 2). Moreover, P1 linkage data indicated that the *ilv-206* marker is only linked 68% to the *rbs-215* marker (Table 3). These data clearly demonstrate the *ilv-206* marker is in the *ilvC* gene and should be designated as *ilvC206*.

Compatible with the enzyme data is our observation that transducing particles are of the

TABLE 1. *E. coli strains and phages used*

Strain or phage	Genotype	Source or reference
Strain		
CU4	F⁻ *galT12* λ⁻	Laboratory collection
CU12	*rbs-215* λ⁻	Laboratory collection
CU393	F⁻ *ilvD2016* λ⁻	Laboratory collection
CU406	F⁻ *galT12 ilvA454* λ⁻	Laboratory collection
CU424	F⁻ *galT12 ilvC462* λ⁻	Laboratory collection
CU431	HfrH Δ(*gal-attλ-bio-uvrB*) *thi* (λ *c*I857 inserted into *ilvC*)	λ *c*1857 lysogenization of KS302
CU437	F⁻ *cya-283*	Laboratory collection
CU477	F⁻ *ilvC206*	Shimada et al. (13) (originally *ilvA206*)
KS302	HfrH Δ(*gal-attλ-bio-uvrB*) *thi*	Shimada et al. (12)
KS505	HfrH Δ (*gal-attλ-bio-uvrB*) *thi* (λ *c*I857 inserted into *ilvC*)	Shimada et al. (12)
UK51	HfrH Δ(*gal-attλ-bio-uvrB*) *thi* (λ *c*I857 inserted into *ilvC*)	λ *c*I857 lysogenization of KS302
UK71	F⁻ *galT12 ilvA454* λ *c*I857 *c*I857 d*ilvADE*	λ transduction of CU406 with UK51 as donor
UK91	F⁻ *cya-283* λ *c*I857 *c*I857 d*ilvC*	λ transduction of CU437 with UK51 as donor
Phage		
λ *c*I857	λ *c*I857 single lysogen, *pm*⁻	Laboratory collection
P1*cm*	21Kb::Tn*9* *clr*100	Rosner (11)
λ ch80*del9*	λ ch80⁺ (*int*)^Δ	Shimada et al. (13)

TABLE 2. *Enzyme levels in several E. coli K-12 derivatives with bacteriophage λ inserted in the ilv cluster*[a]

Strain	Medium	Sp act (μmol/min per mg of protein)				
		TD	DH	AHS	IR	TrB
CU4 (wild type)	Excess	0.013	0.016	0.016	0.005	0.014
	Minimal	0.069	0.035	0.063	0.017	0.033
CU477[b] (*ilvC206*)	Excess	0.014	0.018	0.013	0	0.013
	Lim-Val	0.375	0.072	0.167	0	0.050
	Lim-Ile	1.10	0.372	0.026	0	0.066
	Lim-Leu	0.104	0.068	0.060	0	0.018
KS505 (λ → *ilv*)	Excess	0.024	0.008	0.002	0	0.015
	Lim-Val	0.096	0.042	0.023	0	0.060
	Lim-Ile	0.137	0.101	0.003	0	0.081
CU431 (λ → *ilv*)	Excess	0.029	0.011	0.005	0	0.018
	Lim-Val	0.180	0.060	0.101	0	0.060
	Lim-Ile	0.320	0.209	0.004	0	0.090
UK51 (λ → *ilv*)	Excess	0.021	0.012	0.005	0	0.011
	Lim-Val	0.102	0.046	0.096	0	0.043
	Lim-Ile	0.156	0.101	0.006	0	0.050

[a] The *ilv* enzymes and corresponding structural genes are as follows: TD (*ilvA*), threonine deaminase; DH (*ilvD*), dihydroxy acid dehydrase; AHS (*ilvB* and *ilvHI*), end product-inhibited acetohydroxy acid synthase; IR (*ilvC*), acetohydroxy acid isomeroreductase; TrB (*ilvE*), transaminase B. Cells were grown, cell extracts were prepared, and enzymatic assays were performed as described by Ratzkin et al. (10). Protein concentration was determined by the method of Lowry et al. (8). Val, Valine; Ile, isoleucine; Lim, limited.

[b] This strain was originally designated as CGSC 1421, *ilvA206*.

TABLE 3. *Linkage of the rbs-215 marker with λ-induced mutation(s)*[a]

Donor	Recipient	No. of *rbs*⁺	No. of *ilv*	Linkage to *rbs* (%)
CU477 (*ilv-206*)	CU12 (*rbs-215*)	517	355	68
CU406 (*ilvA454*)	CU12 (*rbs-215*)	476	423	89
CU424 (*ilvC462*)	CU12 (*rbs-215*)	413	251	60

[a] Generalized (P1*cm*) transductions were performed by the procedures of Rosner (11). Rbs⁺ transductants were selected on minimal ribose plus Ilv (isoleucine and valine) plates. The transductants were then scored for *ilv*⁻ character by replica plating onto minimal ribose and minimal ribose plus Ilv plates. The minimal medium was that of Davis and Mingioli (4).

two types described by Shimada et al. (13). One, represented by λd*ilvADE* type, isolated in an Ilv⁺ transductant (UK71) of strain CU406 (*ilvA454*), yielded Ilv⁺ transductants with all *ilvA*, *ilvD*, and *ilvE* mutants tested. The other class, represented by λd*ilvC* isolated in a Cya⁺ transductant (UK91) of strain CU437 (*cya-283*), yielded Ilv⁺ transductants with many of the *ilvC* mutants tested and yielded Cya⁺ transductants with strain CU437.

The site of prophage λ in several such lysogens was determined by the isolation and analysis of

specialized transducing phage from these mutants. All of the λ lysogens tested yielded one or the other of two types of transducing phages (λd*ilvADE* and λd*ilvC*). Although the precise location of the inserted prophages in these strains cannot at present be determined, the insertion sites in other independent lysogens are shown to be identical within the resolution limits of an earlier microscopic study of heteroduplex (7).

Lastly, the key thrust of this short communication is to clarify the discrepancy in the literature on the site of λ insertion in the *ilv* cluster. Shimada and co-workers misclassified the insertion site of strain KS505, apparently owing to an *ilv* mutant incorrectly identified as *ilvA206* rather than *ilvC206*. The reassignment of this *ilv* mutant has now been made as shown in Table 1.

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