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Int-Constitutive Mutants of Bacteriophage Lambda

(lysogenization/regulation/prophage excision/tryptophan synthetase)

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ABSTRACT **The constitutive production of small amounts of *trpB* enzyme in an *Escherichia coli* strain carrying λ*cI*857 prophage within the *trpC* gene has been examined in derivatives of this strain from which portions of the prophage have been deleted. Enzyme production requires a site (p_I) within the prophage close to the left prophage end. Selection for mutants of this lysogen that grow on low concentrations of indole yielded two types of mutations within the prophage: (*a*) *v*2-type, in which all phage genes controlled by the major leftward operator are derepressed; and (*b*) *int-c* type, in which the only phage gene derepressed is *int*. The *int-c* mutations lie in the same part of the prophage as p_I. All *int-c* mutants appear deficient in *xis* gene function, even when derepressed.**

In bacteriophage λ, phage mutations inactivating prophage insertion and excision lie in two genes (*int* and *xis*) close to, and to the right of, the insertion site *att*. They belong to an operon whose transcription is repressible by binding of the *c*I gene product to the operator o_L. A mutation, *sex* 1, that lies close to o_L and reduces transcription of the entire operon, defines the promoter site p_L. Additionally, the product of gene *N* (the first gene of the operon) extends transcription initiated at p_L beyond a stop signal shortly to the left of *N* (refs. 1 and 2; Fig. 1). Protein products of the *int* and *xis* genes have been identified (3), but no biological activity has been demonstrated *in vitro*. It is not known how *int* and *xis* expression are directed toward insertion after infection, excision after derepression, and stabilization in established lysogens.

Study of an abnormal lysogen whose λ prophage is inserted within the *trpC* gene of *Escherichia coli* suggested the existence of a weak consitutive leftward promoter in λ (4). Our present work confirms that *trpB* expression in this lysogen is no longer regulated by the *trp* operator and locates the constitutive promoter (p_I) within λ.

Various evidence suggests that some transcription of *int* (but not *xis*) occurs in the presence of immunity (5) or absence of *N* product (6). It seems plausible, and consistent with the location of p_I, that *trpB* expression results from extension of the constitutive *int* message into adjacent bacterial DNA. To obtain more direct evidence that *int* and *trpB* transcription have a common promoter, we isolated mutant lysogens where *trpB* expression is enhanced: (*a*) *v*2 type, in which transcription from p_L is insensitive to repression and (*b*) a novel type (*int-c*) with the properties expected of a mutation that increases constitutive transcription from p_I.

MATERIALS AND METHODS

Strains. Bacterial strain KS507 (which carries a λ*cI*857 prophage within *trpC*) and its *recA*1 derivative KS1226 are described in ref. 4. Other phage and bacterial mutants are described in ref. 7. Strain KS1226 is a *recA*1 *att*λ$^+$ derivative of KS507.

Media. Tryptone broth (TB) was used for bacterial growth. Phage and bacterial assays were on tryptone agar (TA). The *gal* character was scored on EMB-gal plates. Lysogens were isolated on EMB-O agar. Minimal agar was supplemented with 0.1 μg/ml of biotin, 1% glucose or galactose, and, where required, 20 μg/ml (high-indole agar) or 0.1 μg/ml (low-indole agar) (8, 9). $MgSO_4$ (0.01 M) was used as a diluent.

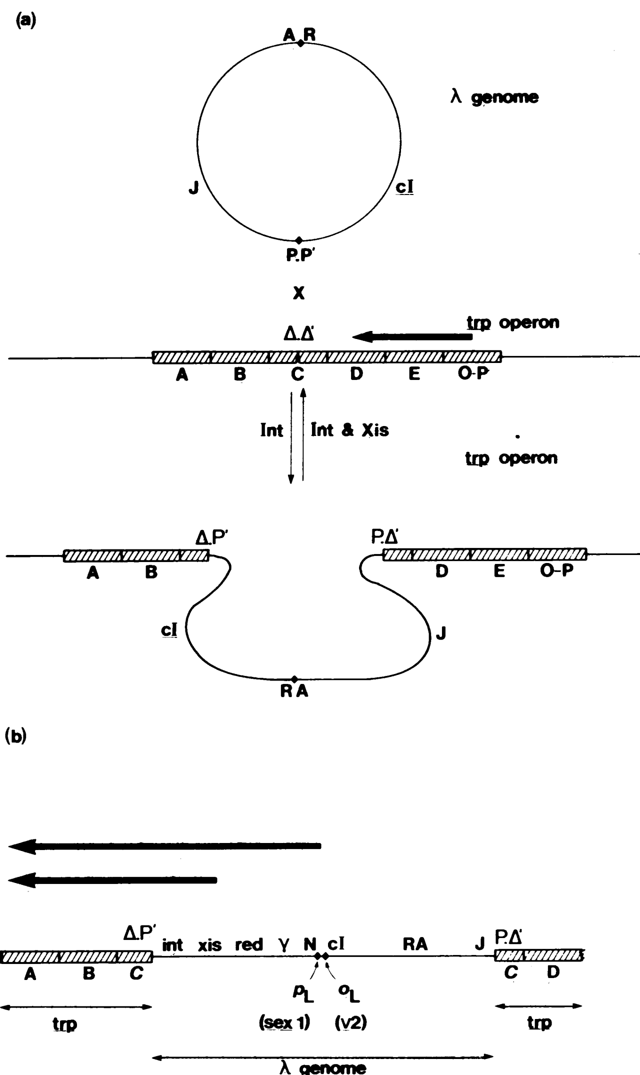

FIG. 1. Insertion and excision of λ prophage within *trpC* (after ref. 4).

Abbreviations: MOI, multiplicity of infection; TB, tryptone broth; TA tryptone agar.

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TABLE 1. *Thermoresistant survivors of* (λ *cI857*)$_{Trp}$

Group	No. of strains	Growth on indole†	Reversion to Trp† on infection by λ‡
A	86 = 65 + 21*	+	+
B	29 = 12 + 17*	+	−
C	2	−	+
D	80	−	−

* These strains were isolated on high-indole plates. The others were isolated on TA.

† +, growth on high-indole plates; −, no growth.

‡ +, appearance of Trp$^+$ revertants; −, no revertants.

Isolation of Thermoresistant Survivors. Strain KS507 was streaked on TA at 30°. After 24 hr, each colony was restreaked on TA or on high-indole agar, and incubated at 41° for 2–3 days. Only one thermoresistant survivor was taken from each streaking; hence each isolate is independent.

Detection of Intact trp Operon. KS507 can revert to Trp$^+$ when Int and Xis functions are supplied by heteroimmune superinfecting phage or induced by heat-pulsing. To test whether thermoresistant derivatives retain an intact *trp* operon, overnight cultures were cross-streaked with λ*cI*857 (about 3×10^{10} per ml) on minimal plates and incubated at 30° for 2 days. Trp$^+$ growth (1–50 colonies) at the intersection means that none of the *trp* operon has been deleted.

Detection of Prophage Genes. λ genes *N*, *O*, *P*, *Q*, *R*, *A*, and *J* were determined (10). To test for gene γ (11), starved cells (0.1–0.2 ml) were mixed with λ*bio*1 (which is Fec$^-$; i.e., unable to form plaques on *recA*$^-$ bacteria) at a multiplicity of infection (MOI) of 3. After 20 min at 33°, 4 ml of TB was added and samples were incubated at 37° for 90 min. $CHCl_3$ was added, and Fec$^+$ recombinants were counted by plating 0.2 ml on a *recA*$^-$ host. A γ^+ strain gives 10–100 Fec$^+$ plaques; γ^- strains gave none.

The γ^- strains were crossed with λ*cI*857 *red*β270 γ210. Strains that generated Fec$^+$ recombinants were classified as *red*$^+$. Usually a *red*$^+$ strain yields 10^3–10^4 Fec$^+$ plaques per 0.1 ml, whereas *red*$^-$ give less than 30.

Presence of an *int* gene (plus *int* promoter) was demonstrated by superinfection curing. Overnight cultures in TB or starved cells (0.1–0.2 ml) were mixed with λ*bio*16 (*int*$^-$*xis*$^+$) at MOI of 2. After 20 min at 33°, 4 ml of TB was added. Cultures were shaken overnight at 37°, then centrifuged, resuspended in 0.4 volume of 0.01 M $MgSO_4$, and aerated 45 min at 37°. These cells (0.1 ml) were plated on minimal plates. Trp$^+$ colonies were counted after 2 days at 37°. Those strains that showed more Trp$^+$ revertants than background (usually around 30–50 Trp$^+$ colonies) were classified at *int*$^+$.

Control infections of λ *int*$^+$*xis*$^+$ to strains of group A (Table 1) gave 1000–2000 Trp$^+$ colonies, whereas λ*bio*69 (Int$^-$Xis$^-$) gave 0–1 Trp$^+$. λ *int*$^+$*xis*$^+$ in group-C strains (Table 1) gave about 300 Trp$^+$ colonies. When the infecting phage carried the *cI*857 mutation, operations were at 30°.

Isolation of trpB Mutants.* Strain KS507 grows on high-indole agar, but cannot form visible colonies in 3 days on low-indole plates. Low-indole agar is therefore selective for mutants that make more tryptophan synthetase (EC 4.2.1.20) than KS507. KS507 or KS1226 was plated on low-indole agar and incubated at 30° for 2–3 days. Colonies appearing on these plates (*trpB** mutants) were purified by restreaking. Only one *trpB** mutant was picked per culture.

Estimation of Int and Xis Function. Exponential cultures of lysogens at 33° in TB supplemented with 0.01 M $MgSO_4$ and 0.2% maltose were infected with λ*att*^{2} *int-am*29 *imm*21 (Int complementation) or λ*att*^{2}*xis-am*6 *imm*21 (Xis complementation) at multiplicities between 0.01 and 0.001. Subsequent procedures were as described (12). A portion of each lysate was diluted 20-fold into 0.01 M EDTA–0.01 M Tris·HCl buffer, pH 7.4, and held at 37° for about 10 min. The solutions were then chilled, and 0.05 volume of 1 M $MgSO_4$ was added. EDTA-treated and untreated lysates were assayed on a λ lysogen of the *gal*$^-$*trpB*$^-$ strain W3623 (13). The numbers in Table 3 are (plaques after EDTA treatment per plaque before EDTA treatment) × 100. The percentages of EDTA-resistant phage in the λ*att*^{2} stocks before infection were 0.2% for *int-am*29 and 0.1% for *xis-am*6.

TABLE 2. *Deletion mapping of* P_I *and trpB enzyme activity*

Group	Strain no.	*trp* genes*		λ genes‡										*trp* genes		*trpB* enzyme‖	
		(*B*)	(*C*)†	*int*	*red*	γ	*N*	*O*	*P*	*Q*	*R*	*A*	*J*	(*C*)§	(*D*)	low *trp*	high *trp*
A	635	+	+	(+)	+	+	(+)¶	−	−	−	−	−	+	+	+	0.40	0.18
	1139	+	+	(+)	+	+	−	−	−	−	−	−	+	+	+	0.43	0.34
	1125	+	+	(+)	+	−	−	−	−	+	+	+	+	+	+	0.27	0.27
	1121	+	+	(+)	−	−	−	−	−	−	−	+	+	+	+	0.31	0.34
C	1115	+	+	(−)	−	−	−	−	−	−	−	+	+	+	+	<0.05	<0.05

* As λ infection yields Trp$^+$ revertants, all *trp* genes must be intact.

† (*C*), left part of *trpC* gene.

‡ See *Methods, Detection of prophage genes*.

§ (*C*), right part of *trpC* gene.

¶ Part of the *N* gene is present.

‖ Cells were centrifuged, washed twice in cold 0.8% NaCl solution, resuspended in Tris·HCl buffer (pH 7.8), and sonicated. Cell debris was removed by centrifugation at 12,000 rpm in a Sorvall refrigerated centrifuge for 25 min. Tryptophan synthetase (TSase B component) was assayed (13); protein was assayed (22). One unit of specific activity is the conversion of 0.1 μmol of substrate in 20 min at 37°/mg of protein. For "high *trp*" extracts, cells were grown at 37° in minimal medium plus L-tryptophan (40 μg/ml), thiamine (1 μg/ml), and biotin (1 μg/ml). Growth flasks were inoculated from overnight broth cultures, and cells were harvested in late logarithmic phase after overnight culture on a shaker. The procedure for "low *trp*" extracts was identical except that only 2 μg/ml L-tryptophan was added.

Curing Frequency. Bacterial lysogenic for λ*cI*857 die at 41° because virus development is induced. The fraction of non-lysogenic cells within a culture can therefore be determined as the ratio of the bacterial titer at 41° to that at 33°. This fraction (assayed on TA) is the curing frequency.

Precise excision of prophage, mediated by Int and Xis, restores the integrity of the bacterial chromosome. For lysogens carrying λ in *trpC*, such excision restores both heat resistance and tryptophan independence. Trp^+ revertants were enumerated on minimal plates at 30°.

To measure spontaneous curing (Table 3), λ*vir*-resistant derivatives were constructed from each *trpB** lysogen. Exponential cultures at 33° were diluted and plated on TA at 33° and 41°. For heat-pulse curing, cultures were diluted into fresh TB at 41°. After 6 min they were returned to 33° for 6–18 hr with shaking, then plated at 33° and 41°.

Phage Release from trpB Lysogens.* Exponentially growing λ*vir*-resistant derivatives were diluted into warmed TB containing 0.01 M $MgSO_4$. After 20 min at 41°, they were incubated 90 min at 37° with shaking and treated with $CHCl_3$. Burst sizes are expressed as phage titer after 90 min of incubation at 37° divided by the colony count at 30° just before the temperature shift.

Enzyme Assays. Crude enzyme extracts were prepared (see legend to Table 2), and the tryptophan biosynthetic enzymes were assayed (13, 14). The same crude extracts were used for λ exonuclease activity assays (kindly performed for us by Dr. V. Simmon) after they were diluted 3-fold with buffer (0.01 M glycine-KOH, pH 9.4). The assay differed from that described (15) only in that we used T7 DNA rather than *E. coli* DNA. The activity (0.11 units/mg of protein) in the control lysogen KS507 may be due to host nucleases or other nonspecific factors.

RESULTS

Mapping of a Secondary Leftward Promoter Within Lambda Prophage. Detection of constitutive *trpB* expression in a strain (KS507) carrying λ within *trpC* suggested the existence of a weak constitutive leftward promoter in λ (4). We tried to locate this promoter (p_I) by deletion mapping. Several independent prophage deletions, isolated among heat-resistant survivors of KS507, were classified into four groups (Table 1). Among 197 Trp^- survivors, 115 could grow on high-indole plates. After infection by λ*cI*857, 86 out of 115 could revert to Trp^+. These 86 strains (group A) were considered to have deletions internal to the prophage. The remaining 29 strains that are $trpB^+$ but cannot revert to Trp^+ (group B) were considered to have experienced deletions that removed some of the *trpC-trpE* region but left the *trpB* gene fused to a functional promoter. Eighty-two Trp^- survivors could not use indole as a tryptophan source. Two of these (group C) could revert to Trp^+ on infection by λ and, thus, have deletions internal to the prophage that remove or inactivate p_I. The remaining 80 strains (group D) cannot revert to Trp^+.

Analysis of residual prophage markers in some of these strains (Table 2), in conjunction with previous data on prophage orientation within *trpC* (Fig. 1), confirms the existence of a leftward promoter within λ, probably between *att* and *red.* Enzyme assays on strains grown at high and low tryptophan concentrations (Table 2) show that (*a*) all group-A strains tested make *trpB* enzyme at a low rate, independent of tryptophan concentration; and (*b*) strain 1115 (group C) forms no detectable *trpB* enzyme under any circumstances. The fact that strain 1115 can be cured to yield Trp^+ derivatives implies that its deletion is internal to the prophage. The absence of enzyme from this strain therefore shows that all of part of p_I lies within the prophage.

Mutations Enhancing Constitutive Expression of trpB. The above results place p_I within that section of λ DNA where the promoter for constitutive *int* expression should lie. If p_I is, in fact, the promoter for *int*, then a single mutation in p_I or any element influencing initiation at p_I should affect transcription of *int* and *trpB* coordinately. Mutants of KS507 with increased *trpB* expression can be selected on low indole. To avoid deletions that simply reconnect *trpB* to a bacterial promoter, we examine only colonies that remain Trp^- and that liberate plaque-forming phage particles after thermal induction. We call these mutants *trpB**. Among eight independent *trpB** isolates, only three exhibited elevated activity of Int. We concentrated our attention on these three and similar strains obtained later and have not examined the biochemical basis of efficient indole utilization by the other five.

Gene Expression in Constitutive Mutants. A mutation that increases leftward transcription from a promoter within the prophage should enhance expression of all phage genes to the left of the promoter site. We thus examined expression of some prophage, as well as bacterial, genes in *trpB** strains.

(*A*) *Int and Xis.* We measured *int* and *xis* expression by using λatt^2 phage (16). After infection of $\lambda att^2 int^-$ phage onto these *trpB** strains at low temperature, three out of eight strains efficiently change EDTA-sensitive $\lambda att^2 int^-$ to EDTA-resistant small phage. One of these three mutants can also change $\lambda att^2 xis^-$ to small phage under the same conditions. Thus, strains 2-26 and 5-18 (Table 3) express only *int* under repression; whereas strain 4-59 expresses both *int* and *xis*. Constitutive expression of *int* and *xis* should also enhance spontaneous prophage loss. Strain KS507 can be cured of λ and simultaneously revert to Trp^+ when Int and Xis functions are supplied by a heteroimmune superinfecting phage (4) or by heat-pulse treatment (Table 3, second row). The rate is low compared to curing of λ prophage at its normal location, presumably because of the bacterial sequences adjacent to the insertion sites.

Frequencies of spontaneous prophage loss by precise excision ("Trp^+ reversion") and by all mechanisms ("λ curing") are summarized in Table 3. The frequency of prophage loss by strain 4-59 was about 500-fold higher than that of KS507. However, this is still 20-fold less than seen after heat-pulse treatment of KS507. Strains 2-26 and 5-18 showed no significant change in spontaneous curing. These results confirm that strain 4-59 expresses *int* and *xis* under repression, whereas *xis* expression by strains 2-26 and 5-18 is undetectable.

Transient heat-pulse treatment greatly increased prophage loss from KS507, 4-59, and $recA^-$(λ*I*857), but had little effect on strains 2-26 and $recA^-$(λ*int-c cI*857) (Table 3). This suggests that these strains fail to express *xis*, even when derepressed. Phage production after thermal induction provides another measure of Int and Xis function (Table 3, last column). Whereas KS507 and 4-59 produce about 6×10^{-3} phage per induced cell, strains 2-26 and 5-18 produced 300-fold less. This again suggests a severe defect in Xis function,

TABLE 3. *Properties of trpB* mutants*

Strain†	Heat-pulse treatment	Complementation in att[2] test		Frequency of excision‡		Average burst size
		Int	Xis	Trp$^+$ revertants	λ curing	
KS507	−	1.1	5.3	$<3 \times 10^{-8}$	5.6×10^{-8}	NT
	+	79	71	3.2×10^{-4}	3.2×10^{-4}	6×10^{-3}
2-26	−	95	6.6	$<3 \times 10^{-8}$	1.2×10^{-7}	NT
	+	NT§	NT	1.9×10^{-7}	5.9×10^{-7}	0.023×10^{-3}
5-18	−	71	4.1	$<3 \times 10^{-8}$	1.6×10^{-7}	NT
	+	NT	NT	NT	NT	0.020×10^{-3}
4-59	−	52	36	1.5×10^{-5}	1.9×10^{-5}	NT
	+	NT	NT	5.7×10^{-2}	5.7×10^{-2}	10×10^{-3}
recA$^-$ (λ*cI*857)	−	NT	NT	NT	2.4×10^{-6}	NT
	+	NT	NT	NT	3.8×10^{-1}	36×10^{0}
recA$^-$ (λ*cI*857 *int-c*)	−	NT	NT	NT	8.7×10^{-4}	NT
	+	NT	NT	NT	5.1×10^{-3}	0.13×10^{0}

† Strains 2-26, 5-18, and 4-59 are spontaneous *trpB** mutants of KS507. The last two strains are strain KS142 (a *recA*$^-$ derivative of strain W3623) lysogenized with (*a*) wild-type λ*cI857*; (*b*) a phage recombinant derived by crossing a *trpB* transducing phage derived from 2-26 with λ*h bio*11 *cI*857 and isolating P.P′ *int-c* recombinants. The last two lysogens thus carry the same prophages as do KS507 and 2-26, respectively, but these prophages are inserted at the normal attachment site rather than within *trpC*.

‡ See *Methods, Curing frequency*.

§ *NT* = not tested.

even in absence of repression. This excisionase deficiency is also seen in a lysogen carrying a prophage derived from strain 2-26 but inserted at the normal λ attachment site (Table 3, last row).

We isolated three additional *int*-constitutive lysogens from KS507 and two from KS1226. All five were deficient in Xis function. The mutations (*int-c*) that distinguish strains 2-26 and 5-18 from KS507 thus might constitute changes in p_I. Their observed excisionase deficiency under derepression was unanticipated. Strain 4-59 has different properties and (Shimada and Campbell, in manuscript) is indistinguishable from a mutation (*v*2) of the operator controlling transcription from the major leftward promoter p_L (17).

(*B*) *Trp enzyme activities.* Mutations increasing transcription from either p_I or p_L should increase transcription of genes of the *trp* operon to the left of the inserted prophage (Fig. 1), and this transcription should be unaffected by tryptophan. Those *trp* genes to the right of the prophage should remain under control of the *trp* operator. Assays of the parent strain KS507 and one *trpB** mutant of each type (*int-c* or *v*2) for enzymes determined by a gene to the left (*trpB*) and one to the right (*trpE*) give results consistent with these expectations

TABLE 4. *Trp enzyme activity of trpB* lysogens*†

Strain	*trpB* enzyme activity			*trpE* enzyme activity		
	low *trp*	high *trp*	dere-pression ratio	low *trp*	high *trp*	dere-pression ratio
KS507	0.32	0.35	0.92	2.1	0.021	100
2-26	5.5	5.3	1.0	1.1	0.012	92
4-59	2.6	2.7	0.96	2.1	0.024	88

† Crude enzyme extracts were prepared as in Table 2 (legend) except that cultures were incubated at 30°. The *trpB* enzyme, *trp* synthetase (TSase B component), was assayed as in Table 2. The *trpE* enzyme, anthranilate synthetase, was assayed as described (14).

(Table 4). The specific activity of *trpB* in the *int-c* strain 2-26 is 17-fold higher than that of wild-type *E. coli* under *trp* repression, and about $^1/_3$ of that under full derepression.

(*C*) λ *exonuclease activity.* λ exonuclease (product of one of the *red* genes, Fig. 1) was measured using the same extracts prepared for *trp* enzyme assays. The activities of KS507 and 2-26 extracts were, respectively, 0.11 and 0.065 units per mg of protein, whereas the 4-59 extract contained 0.93 units/mg of protein. The exonuclease activity was unaffected by *trp* repression. Thus, only the *v*2-type lysogen showed significant exonuclease in the presence of immunity as expected if the *int-c* mutation influences transcription from a promoter located to the left of *red*.

(*D*) *Phage stocks carrying int-c or v2-like mutations.* Studies on phages produced from *trpB** strains (Shimada and Campbell, in manuscript) show that (*1*) the *v2*-like mutation causes constitutive expression of *N*, *red*-γ, *xis* and *int*, whereas *int-c* derepresses only *int*; (*2*) the *int-c* mutation maps within phage DNA at or close to p_I and (*3*) in double lysogens, the effect of *int-c* on *trpB* is manifested only in *cis*.

DISCUSSION

The constitutive production of small amounts of tryptophan synthetase in bacteria carrying a λ prophage within *trpC* led to the postulation of a leftward, immunity-insensitive promoter (p_I) within λ. Retention of constitutive synthesis by derivatives of such bacteria from which parts of the prophage have been deleted localize this promoter within the prophage, somewhere between *att* and *red* (Fig. 1*b*). In light of work

TABLE 5. *Gene expression by mutants in presence of repressor*

Phage	Amount of gene expression				
	trpB	*int*	*xis*	red or γ	*N*
Wild type	weak	weak	−	−	−
int-c	+	+	−	−	−
*v*2-type	+	+	+	+	+

showing that the *int* gene is expressed to some extent in a repressed prophage, it seems plausible that p_I is a promoter for constitutive transcription of *int*. This hypothesis is corroborated by the properties of mutants selected for enhanced constitutive tryptophan production. Two types of phage mutations have been found in such mutants. (*a*) *int-c* mutations, located at or near p_I, and (*b*) *v*2-type mutations, whose phenotype matches that of previous isolates that affect the major leftward operator o_L.

Under repression, where most phage functions are turned off, these mutants have the phenotypes shown in Table 5. These results fit the idea that *int-c* mutations increase transcription from a promoter that normally controls only *int*. Alternatively, the *int-c* mutations might not affect transcription from the wild-type *int*-gene promoter, but create a new promoter or activate a promoter with another normal function.

Excisionase Defect of int-c Mutations. Table 5 shows the phenotypes of *int-c* and *v*2-type mutants under repression. When wild-type prophage is derepressed, all the functions listed are activated. *Int-c* mutants, however, show little excisionase activity even when derepressed (Table 3). This deficiency can be remedied by an xis^+ prophage in *trans* (Shimada and Campbell, in manuscript), suggesting that Xis is not simply inactivated by excess Int. Fig. 1 illustrates a possible mechanism: that p_I or its operator lies within the *xis* gene, so that mutation of these elements affects the structure of the Xis protein.

Significance of Secondary int Promoter. Each advance in our knowledge of the interaction between phage and bacterial chromosome emphasizes that insertion and excision do not proceed haphazardly but in a highly regulated manner. Lysogenization might have involved recombination between identical base sequences of viral and host DNA, catalyzed by host recombinases; instead, the virus breaks and joins DNA sequences whose homology, if any, is too slight to allow general recombination. Excision might have come about by a simple reversal of insertion; instead, a separate gene product is required only for excision. This permits regulation of the direction, as well as the extent, of the reaction.

Various authors (5, 18–21) have shown that maximal complementation for *int* function is not provided by an int^+ phage under repression, and that N^- phages express *int* poorly. The straightforward molecular interpretation is that most transcription of *int* is initiated at p_L, and therefore that *int* and *xis* are usually cotranscribed. Some authors also observed limited expression of *int*, but not *xis*, under immunity. It was not known whether this indicated a second pathway that allowed transcription of *int* without *xis* or merely reflected an inability to detect small amounts of *xis*. As our present results require a second promoter in any case, the former alternative is favored.

Obligatory cotranscription of *int* and *xis* would leave unexploited the potentiality for transcriptional control over the direction of the insertion-excision reaction. Weisberg and Gottesman (12) showed that the ability of a heat-pulsed λ*cI*857 lysogen to complement a superinfecting $\lambda att^2 xis^- imm21$ phage decayed more rapidly than ability to complement $\lambda att^2 int^- imm21$. Thus, differential *in vivo* stability of *int* and *xis* products might be the principal factor controlling the direction of the reaction. Transcription originating at p_I may also have contributed to their result.

The most obvious function for a secondary *int* promoter would be to direct the insertion-excision reaction toward insertion, thereby favoring either establishment or maintenance of stable lysogeny. The fact that lysogens express *int* constitutively suggests that stabilization is at least one of its functions. A role in prophage establishment would be more credible if transcription from p_I were controlled by phage-specified products. Location of the secondary promoter within the *xis* gene, if verified, might allow further refinement of the pattern of control.

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