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DNA sequence of regulatory region for integration gene of bacteriophage λ

(promoter structure/RNA regulation/bacteriophage λ cII/cIII proteins)

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ABSTRACT **The cII and cIII proteins specified by bacteriophage λ direct the lysogenic response to infection through the coordinate establishment of repression and integration of the viral DNA. The regulatory activity of cII/cIII involves positive regulation of two promoter sites: the p_E promoter, turning on expression of the cI protein that maintains lysogeny, and the p_I promoter, activating synthesis of the Int protein for integrative recombination. Regulation of the p_I promoter provides for differential expression of the Int protein with respect to the excision-specific Xis protein from the closely linked *int* and *xis* genes. We have determined the DNA sequence of the p_I promoter region for wild-type λ DNA and for two classes of mutations: *intc* mutations, which result in a high rate of Int synthesis in the absence of cII, and deletion mutations, some of which eliminate cII-activated expression of the *int* gene. We find a sequence with considerable homology (11 of 15 bases) to a "typical" (computer-generated) promoter sequence, adjacent to a region with striking homology (11 of 14 bases) to part of the p_E promoter region. This presumed p_I sequence overlaps the start of the *xis* gene and includes the site of two *intc* point mutations. A cII-insensitive *xis*$^+$ deletion partially removes the proposed p_I sequence; a deletion that leaves the p_I sequence intact but terminates 21 bases upstream does not interfere with cII activation of the *int* gene. From our results and the analysis of the p_E region, we suggest that cII acts in the promoter −35 recognition region to facilitate binding by RNA polymerase at the −10 interaction region. Differential expression of the *int* and *xis* genes results because the p_I transcript lacks the initiation codon for Xis protein synthesis.**

Bacteriophage λ provides a conveniently studied example of a developmental switch in which a genome can specify a choice of responses along alternative pathways. For λ the choice is binary: between the productive pathway that produces lysis of the host cell and new virus particles and the lysogenic pathway that results in an integrated, repressed prophage (1–4). After infection of a host cell, the cII/cIII proteins of λ are the principal regulatory elements determining the frequency of the lysogenic or productive responses. To carry out this "switch function," cII/cIII provide for three regulatory events: activation of synthesis of cI protein, the repressor that maintains lysogeny (5, 6); activation of synthesis of Int protein, required for insertion of the phage DNA into the host chromosome (7–10); and decreased synthesis of the late proteins of the productive pathway (11, 12) (Fig. 1).

The Int protein functions both in the insertion of viral DNA into the host chromosome during lysogenization and in the reverse process (excision) that precedes virus production by a lysogenic bacterium. Excision requires not only the Int protein but also a second viral protein, Xis, which is not needed for insertion. Differential regulation of *int* and *xis* can thus control the direction of the reaction at the appropriate times in the viral life cycle. The general nature of this differential regulation is illustrated in Fig. 1. The *int* gene can be transcribed from two promoters, p_I and p_L. Transcription from p_L, resulting in expression of both *int* and *xis*, takes place in derepressed lysogens and also in infected cells. Transcription from p_I, which is activated by cII/cIII, results in production of Int alone (7–10). By employing the same macromolecular regulators to turn on both the *cI* gene and the *int* gene, the phage assures that those infected cells choosing the lysogenic response will express both functions (repression and insertion) needed to achieve stable lysogeny.

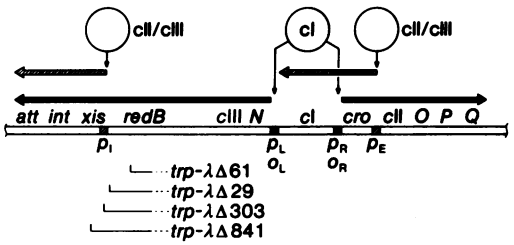

FIG. 1. Regulation of transcription during establishment of lysogeny by λ. The cII and cIII proteins stimulate transcription (hatched arrows) from two promoters: the integration promoter p_I and the establishment promoter p_E. Transcription from p_I results in expression of the *int* gene; Int protein is required for integration of the phage DNA into the host chromosome. Transcription from p_E gives expression of the *cI* gene; cI protein binds to the operators o_L and o_R, shutting off the λ early transcripts (filled arrows) from p_L and p_R. The end points of the deletions caused by the λ*trp* fusions *trp*-λΔ61, *trp*-λΔ29, *trp*-λΔ303, and *trp*-λΔ841 are shown below the λ map.

Recent efforts to understand the regulatory role of cII/cIII have been directed toward the mechanism for the positive transcriptional regulation of the *cI* and *int* genes. For *cI* gene expression, a cII-controlled‡ promoter site p_E between the *cro* and *cII* genes was inferred from an analysis of *cis*-dominant mutations that block the capacity of cII to activate the *cI* gene (*cy*$^-$ mutations) (5, 6, 14). This promoter site has been established more rigorously recently by a deletion analysis (15) and by the isolation of the p_E-specified RNA (16). The results of combined genetic and sequence analysis suggest two functional regions for p_E, defined by two clusters of mutations, *cyl* and *cyr*

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‡ Although both the cII and cIII proteins are involved in regulating the establishment of repression, cII probably has the more direct role (e.g., see ref. 13); for simplicity, in the remainder of the text the regulatory interaction will be designated by cII only.

(17). These two regions, termed here p_{El} and p_{Er}, may define, respectively, the site of interaction with RNA polymerase and the site of cII action (16, 17) (see *Discussion*).

The cII-regulated promoter for *int* gene expression, p_I, has been less well characterized than p_E. Two lines of evidence have indicated that p_I is close to or within the λ *xis* gene. First, the *xis* gene is not subject to cII activation (8, 18). Second, point mutations have been isolated that allow a high rate of Int expression in the absence of cII (*intc* mutations) (19); the mutations produce an Xis⁻ phenotype *in vivo* (19) and a newly active promoter site *in vitro* (20). These results suggest that p_I and *xis* might overlap. On the other hand, a deletion analysis of the λ *int-xis* region, carried out by fusing this region to the tryptophan operon promoter, has indicated that at least part of p_I lies to the right of *xis;* one deletion (*trp*-λΔ303) eliminates the cII activation of p_I but a functional *xis* gene is retained (21) (Fig. 1).

In an effort to understand the structure and regulation of the p_I promoter, we have determined the DNA sequence of the p_I region for wild-type phage, for three λ*trp* fusions, and for two *intc* mutations. We have found a sequence that exhibits considerable homology with a typical promoter sequence, adjacent to a region that shares considerable homology with the p_{Er} region and which is essential for cII-dependent transcription *in vivo*. This presumed p_I sequence overlaps the start of the *xis* gene, is partially deleted in the *xis*⁺, cII-insensitive *trp* fusion, and includes the site of the two *intc* point mutations. We suggest that cII facilitates binding and initiation by RNA polymerase at the p_I promoter. Results and interpretations similar to ours have been derived by Hoess *et al.* (22).

MATERIALS AND METHODS

Bacteria and Bacteriophage. The *Escherichia coli* strains used and their relevant genetic characteristics were: N1625 (ligase overproducer) (23), C600Su⁻ (nonsuppressor) (11), W3110*trp*ΔEA2 (deleted for the entire *trp* operon) (24), and LH114, LH122, LH156, and MB112, carrying the λ*trp* fusions *trp*-λΔ61, *trp*-λΔ841, *trp*-λΔ303, and *trp*-λΔ29, respectively (ref. 21; this work). The phage point mutations used were: *cIts*857 (25), *Sam*7 (26), *intc*262 and *intc*518 (19), and *xisam*6 (27). λ*trp*503, a transducing phage that carries the *E. coli trp* operon, was constructed by K. Shimada.

Construction of Plasmids Carrying λ*trp* Fusions. Each of the λ-*trp* fusion strains LH114, LH156, LH122, and MB112 was lysogenized with λ*trp*503. Integration of the phage occurs within the λ-*trp* fusion region by homologous recombination (Fig. 2*a*). These lysogens were induced, and individual plaques from the resultant lysates were tested. Those isolates that had lost the ability to transduce W3110*trp*ΔEA2 to growth on unsupplemented minimal medium were presumed to have been excised from the λ*trp*503 lysogens in such a way as to include the λ-*trp* fusion region (Fig. 2*b*). In this manner, phages carrying each of the λ-*trp* fusions were constructed.

The fusion regions were then transferred to the plasmid pDB106 *in vivo* as follows: λ-*trp* fusion phage were adsorbed to cells of strain W3110*trp*ΔEA2 (pDB106) at one phage per cell at 37°C for 15 min. The cells were diluted 1:10 with prewarmed L broth (28) and incubated at 37°C for 90 min. Cell debris was removed by centrifugation, protein was extracted with phenol, and nucleic acids were precipitated with ethanol. The last two steps were repeated. The DNA was then directly used to transform competent cells of strain W3110*trp*ΔEA2 by the method of Cohen *et al.* (29). In each case, 0.5–3% of the ampicillin-resistant transformants were able to grow on indole medium (19). We assumed that the plasmids in these strains had arisen by a recombination event that had inserted the λ-*trp*

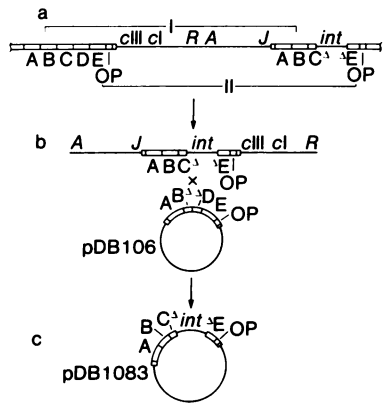

FIG. 2. Construction of λ*trp* plasmid pDB1083. A λ*trp*503 lysogen of strain LH156, in which the prophage is inserted into the λ*trp* fusion region (*a*), was induced by temperature shift to 43°C. Excision of the induced prophage by recombination between homologous sequences could occur in either of two ways: the first (I) would regenerate the λ*trp*503 phage; the second (II) produces the λ*trp* phage carrying the *trp*-λΔ303 fusion region. This latter phage (*b*) was crossed with plasmid pDB106, a derivative of pBR322. Recombination between the promoter-proximal and promoter-distal portions of the *trp* operon carried on both plasmid and phage results in the insertion of the *trp*-λΔ303 fusion region of the phage into pDB106, producing plasmid pDB1083 (*c*). Plasmids pDB1086 (carrying the fusion *trp*-λΔ61), pDB1088 (carrying *trp*-λΔ841), and pDB1089 (carrying *trp*-λΔ29) were derived in the same fashion from λ*trp*503 lysogens of LH114, LH122, and MB112, respectively.

fusion region of the phage (including an intact *trpB* gene) into the plasmids (Fig. 2*c*). This assumption was confirmed by detailed restriction analysis of the plasmid DNAs. Plasmids containing the fusions *trp*-λΔ61, *trp*-λΔ303, *trp*λΔ841, and *trp*-λΔ29 were designated pDB1086, pDB1083, pDB1088, and pDB1089, respectively.

When tested by the described method (21), pDB1086 exhibited cII-activated Int function, whereas pDB1083 showed only cII-independent *int* and *xis* gene expression.

The pDB106 plasmid used in these studies was constructed *in vitro* from two derivatives of the pBR322 plasmid carrying different portions of the *E. coli trp* operon. (These plasmid strains were kindly provided by G. Zurawski and B. Nichols.) DNA prepared from these plasmids was cleaved with *Sal* I and *Hin*dIII restriction endonucleases, and the digests were mixed and ligated with T4 polynucleotide ligase. One product of the ligation was pDB106, which was shown by restriction analysis to carry the proximal and distal portions of the *trp* operon fused centrally at the *Hin*dIII endonuclease cleavage sites located in *trpB* and *trpD*.

Preparation, Restriction Mapping, and Sequence Determination of DNA. λ*intc*262*c*I857*Sam*7, λ*intc*518*c*I857*Sam*7, and λ*xis*6*c*I857*Sam*7 were grown by infection of C600Su⁻ as described (10), except that the medium contained 1% Difco tryptone, 0.5% Difco yeast extract, and 0.5% NaCl, with no added ^{32}P-labeled inorganic phosphate. λ*c*I857*Sam*7 was obtained by thermal induction (15 min at 42°C) of the lysogen N1625(λ*c*I857*Sam*7) growing in the above medium, followed by incubation for 3 hr at 37°C. The procedures for cell lysis, concentration of the phage, and extraction of the DNA were as described (10). Plasmid DNA was prepared by the method of Clewell and Helinski (30).

Restriction endonuclease cleavage sites were mapped as described by Kamp *et al.* (31). Restriction fragments were isolated as described (20). Subsequent procedures for determination of the DNA sequence of restriction fragments were as described by Maxam and Gilbert (32, 33).

Enzymes and Other Materials. Restriction enzymes were purchased from New England BioLabs. Alkaline phosphatase was obtained from Worthington or Boehringer Mannheim, T4 polynucleotide kinase was from P-L Biochemicals, and [γ-^{32}P]ATP (>2000 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was from ICN.

RESULTS

DNA Sequence of p_I Region. We first constructed restriction maps of the DNA surrounding the p_I region of λ and the λ-*trp* fusions by using *Hha* I, *Hin*cII, *Hae* III, and *Taq* I (data not shown). Then we determined the sequence of the appropriate fragments. The sequence of wild-type p_I is shown in Fig. 3. This sequence contains three features of obvious interest. First, there is a region (bases 1339–1353) that shows extensive homology (11 of 15 bases) with a computer-generated typical sequence for the RNA polymerase "interaction" site of a promoter (34). The interaction region is often characterized by the seven-base "Pribnow box" (5′-T-A-T-Pu-A-T-Pu-3′) (35); one example of a sequence similar to this is marked by the thick line on Fig. 3.

A second notable feature of the sequence is a region (bases 1360–1373) with extensive homology (11 of 14 bases) to the p_{Er} region, identified as a possible recognition site for cII regulation of the *cI* gene (17). Taken together, these two features suggest that cII might act in the p_{Er}-homologous, righthand boxed sequence to promote interaction by RNA polymerase at the lefthand boxed sequence. Additional data in support of this concept are presented below.

A third feature of the sequence is a region (bases 1375–1394) that exhibits marked similarity to sequences sometimes found at the termination of transcription: six thymidine residues, preceded by a partially symmetric sequence that can fold into a stem-and-loop structure (36). Although this aspect of the sequence, taken alone, leaves open the possibility for regulation by antitermination, other considerations argue strongly against this mechanism (see *Discussion*).

Location of Deletion End Points of λ*trp* Fusions. The cII-dependent promoter p_I can be localized by analysis of deletions that either eliminate or leave unchanged the ability of cII to stimulate *int* expression. In strains bearing the *trp*-λΔ303 and *trp*-λΔ841 fusions, *int* expression is not turned on by cII. Therefore, these fusions eliminate at least part of p_I (ref. 21; Fig. 1). On the other hand, the *trp*-λΔ29 fusion allows typical cII-dependent expression of *int* (data not shown). Therefore, some elements of the p_I promoter, perhaps including the cII-recognition region, lie between the fusion points of *trp*-λΔ303 and *trp*-λΔ29. We have determined the DNA sequence surrounding the fusion points (Fig. 3). The region deleted by *trp*-λΔ303 includes most of the region homologous to p_{Er}; this result supports the identification of the righthand boxed sequence of Fig. 3 as an element of the p_I promoter. The region deleted by *trp*-λΔ29 begins only 21 bases upstream of the righthand boxed sequence; thus, the p_I promoter and cII recognition site cannot extend much to the right of the boxed sequence.

DNA Sequence Changes for *intc* Mutations. The *intc* point mutations provide for constitutive expression of the *int* gene *in vivo* (19); *in vitro* these mutations provide for an RNA polymerase binding site and transcription start in the p_I region not found in wild-type λ (20). Thus, the *intc* mutations probably generate a promoter that is active in the absence of cII.

We have determined the DNA sequence change for two of the *intc* mutations, *intc*262 and *intc*518. Both mutations were found to be G·C → A·T base pair changes at position 1345 (Fig. 3). This change lies within the presumptive interaction site for RNA polymerase outlined in Fig. 3, and it brings the site into better agreement with the consensus sequence (34, 35). Hoess *et al.* (22) have found that the *intc*226 mutation is also a G·C → A·T base pair change at position 1345.

The location and properties of the *intc* mutations indicate that the surrounding boxed sequence is indeed a potential polymerase interaction site. At least two interpretations are possible: (*i*) the boxed sequence is part of the p_I promoter, which is activated by cII in wild type, but is rendered constitutive by

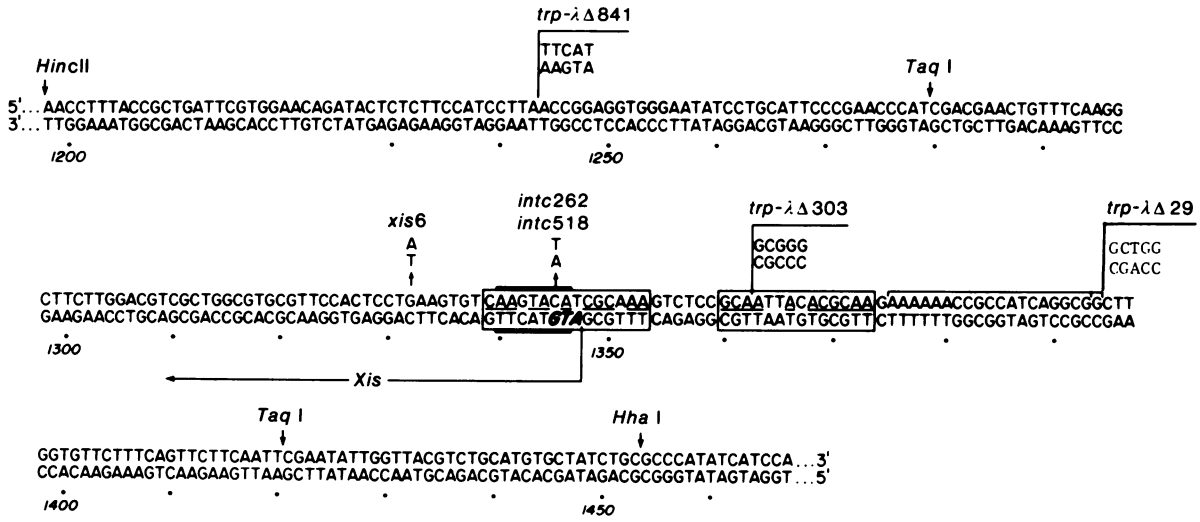

FIG. 3. DNA sequence of p_I promoter region and fusion end points of *trp*-λΔ29, *trp*-λΔ303, and *trp*-λΔ841. The bases are numbered relative to the center of the common core of the attachment site (22). Some of the restriction sites used to generate fragments for sequence determination are also shown. The junctions between λ and *E. coli trp* DNA in fusions *trp*-λΔ29, *trp*-λΔ303, and *trp*-λΔ841 lie at bases 1394, 1362, and 1243, respectively. In each case, the first five nucleotides of *trp* DNA to the right of the fusion point are shown for comparison. The box enclosing bases 1360–1373 indicates a sequence showing considerable homology to the proposed cII interaction site of the p_E promoter (17); the homologous bases have been underlined. The boxed region from base 1339 to base 1353 represents a possible RNA polymerase interaction site; the bases within this region that agree with a consensus sequence for such interaction sites (34) have been underlined. The area within this region marked by heavy lines (bases 1340–1346) represents a second type of common sequence noted at the interaction site, the Pribnow box. The start codon of the Xis protein is shown in italics, and the coding region for Xis is indicated below the DNA sequence. The line above bases 1375–1394 indicates a possible transcription termination signal. The base changes of the mutations *intc*262, *intc*518, and *xis*6 are also shown.

the G·C → A·T base change at position 1345; or (*ii*) the boxed region represents a sequence unrelated to the p_I promoter, which is by chance a potential RNA polymerase interaction site, turned into an active promoter by the *intc* base change. Because of the close proximity of this polymerase interaction site to the p_E-homologous sequence, we favor the first interpretation. We suggest that the presumed polymerase interaction and cII recognition regions boxed in Fig. 3 define two segments of the p_I promoter, p_{II} and p_{Ir}, analogous to the p_E promoter (see *Discussion*).

Location of Start Codon of *xis* Gene. The *trp*-λΔ303 fusion eliminates the ability of cII to activate p_I, but leaves the *xis* gene functional (21), indicating that at least part of p_I lies upstream from the *xis* gene. However, there is little or no *xis* gene expression from p_I (8, 18). One possible explanation for these results is an overlap between p_I and the start of the *xis* gene, such that the p_I transcript contains only a portion of *xis*. This explanation predicts that the start codon of the *xis* gene lies within the p_I promoter sequence.

In order to establish the startpoint of the *xis* gene, we have determined the sequence change for the nonsense mutation *xis*6. The mutation is a C·G → T·A base pair change at position 1332 of Fig. 3, generating a UAG stop codon in the RNA for the *xis* gene (lower DNA strand in Fig. 3). The first potential start codon upstream from this translation stop signal, in the same reading frame, is the AUG coded for by bases 1347–1345, shown in italics in Fig. 3. No other AUG or GUG in-phase sequences exist between this possible start and an in-phase stop signal (UGA) at positions 1389–1387. Furthermore, the *trp*-λΔ303 fusion at position 1362 allows *xis* gene expression and therefore presumably does not enter the *xis* gene. Thus, the AUG at positions 1347–1345 should define the start of the *xis* gene. This probable start signal for *xis* translation lies partially within the Pribnow box of our proposed promoter sequence. Because transcription typically initiates about 10 bases downstream from the center of the Pribnow box of a promoter, an RNA initiated from our proposed promoter sequence should begin near bases 1333 and 1334, and therefore would not contain the first five codons of the *xis* gene. The proposed overlap between the p_I promoter and the *xis* gene would provide for differential synthesis of Int vs. Xis proteins during the lysogenic response (see *Discussion*).

We have analyzed the RNA synthesized *in vitro* by RNA polymerase from an *intc* DNA template (20). Our results indicate that the RNA start is nine bases downstream from the center of the proposed Pribnow box containing the *intc* mutations (unpublished results). Therefore this region can serve as the polymerase interaction site of a promoter.

The AUG start codon for *xis* overlaps the site of the *intc* base change. The *intc* mutations thus produce an Xis⁻ phenotype through alteration of the initiation codon for translation of the *xis* gene.

DISCUSSION

Structure and Regulation of p_I Promoter. We have identified a DNA sequence that we believe to be the promoter p_I for cII-regulated expression of the *int* gene. The proposed promoter consists of two regions, designated p_{II} and p_{Ir}. We identify p_{II} with the RNA polymerase interaction site for two primary reasons: (*i*) there is striking homology between this region and a "consensus" sequence for interaction sites (34); and (*ii*) the *intc* mutations that generate a constitutive promoter site *in vitro* lie within p_{II} and bring this sequence into better agreement with the consensus sequence. Deletion mapping establishes that p_{Ir} is an integral part of the cII-controlled promoter. In particular, *trp*-λΔ29 allows cII-dependent gene expression, whereas *trp*-λΔ303, which removes part of p_{Ir}, does not. Based on evidence so far, the *trp*-λΔ303 fusion might abolish promoter function, cII-activation, or both. We identify p_{Ir} with the site for cII regulation because of the impressive homology with the proposed region for cII regulation of the p_E promoter (p_{Er}) (ref. 17; Fig. 4).

The structural organization of the proposed p_I promoter is similar to that of p_E (ref. 17; Fig. 4). In both cases, the proposed cII regulation site lies about 35 base pairs upstream from the startpoint of transcription, in a region thought to be generally important for RNA polymerase recognition of promoter sequences (38). The presence of the proposed cII regulation site within this region suggests that cII might facilitate the binding of polymerase at p_{II} and p_{EI} (−10 region); the mechanism might involve a cII interaction with polymerase or a cII-mediated alteration in DNA structure. This arrangement of the sites for positive regulation and polymerase interaction apparently differs from that found in the *lac* operon, in which the catabolite repressor protein/cyclic AMP complex binds in the −60 region, adjacent to the recognition site, to activate the *lac* promoter (39). However, the catabolite repressor protein/cyclic AMP site is within the recognition region for the *gal* operon (40); thus *lac* may be an exception for positive regulation.

The possible transcription termination signal found in the p_I region (Fig. 3) suggests a potential alternative mechanism for cII regulation, in which cII acts to antagonize the termination of transcripts initiated upstream, allowing them to extend through the terminator into the *int* gene (41). However, this proposed antitermination mechanism is inconsistent with the

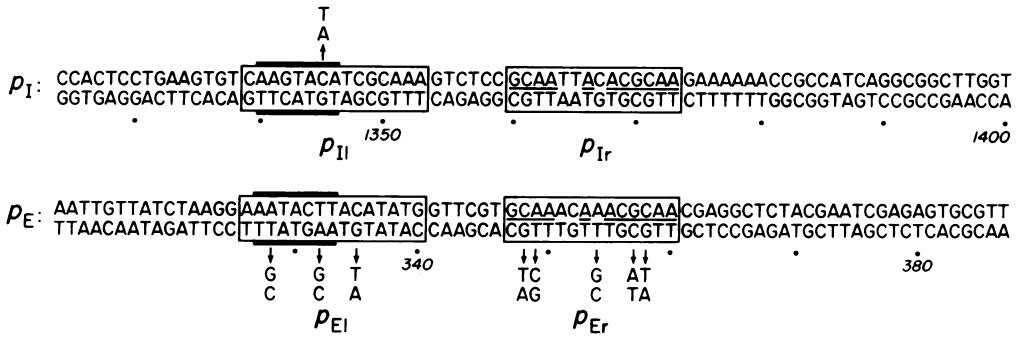

FIG. 4. Comparison of DNA sequences in p_I and p_E promoter regions. Bases of the p_I region are numbered as in Fig. 3, and bases of p_E are numbered as in ref. 37. Right-hand boxed areas indicate proposed cII interaction sites of the two promoters; homologous bases within the two areas are underlined. Left-hand boxed sequences represent the proposed polymerase interaction regions of the two promoters, and subsequences marked by heavy lines represent the Pribnow box sequences. The base change indicated in the p_I sequence is that found in mutations *intc*262 and *intc*518. Base changes shown in the p_E sequence are found in the *cy*⁻ (p_E-defective) mutations (from left to right) 3048, 2001, 3019, 3003, 42, 844, 3008, and 3001 (see ref. 17).

regulatory properties of wild-type and mutant strains. First, it fails to explain the observation that the *xis* gene is not subject to cII regulation, because the extended transcripts should contain all of the sequences necessary to allow translation of the *xis* gene. Second, the *trp*-λΔ29 fusion eliminates both termination events between the *trp* promoter and the *int* gene and also the leader sequence which would be antiterminated, but leaves cII regulation intact (unpublished results). The termination sequence might instead function to protect the prophage from "inappropriate" transcription of the *int* and *xis* genes; expression of Int and Xis in the absence of induction would allow excision of the prophage without expression of the genes for lytic development, resulting in loss of the viral genome.

Regulation of *int-xis* Region. Recent studies indicate that DNA inserting elements are widespread among prokaryotes and perhaps eukaryotes as well (42). Bacteriophage λ is the best characterized system in terms both of the enzymology of the reactions and of their genetic control. Wherever such insertion functions play a biologically meaningful role in the life of the element or of its host, it may be advantageous to regulate both the rate and the direction of the process. The dual control of the *int-xis* segment from p_L and p_I provides a satisfying example of how insertion and excision functions respond to specific signals at appropriate times in the viral life cycle.

Besides providing some insight into the fine structure of the p_I promoter, the present data verify one feature of the system that has no teleological rationalization. Rather than lying between the *int* and *xis* genes, the p_I promoter overlaps the beginning of the *xis* gene so that almost the entire *xis* sequence is transcribed as a translationally inactive leader segment of the p_I message. This arrangement might result from optimization for regulatory aspects as yet undefined; for example, some translational regulation in this region is indicated by the observation that the p_L transcript produces Xis but very little Int (7–10). Alternatively the location of p_I might reflect the evolutionary history of this DNA segment (43).

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