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Gene regulation on broad host range plasmid RK2: identification of three novel operons whose transcription is repressed by both KorA and KorC

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ABSTRACT

The product of the korA gene of broad host range plasmid RK2 is a key transcriptional repressor which regulates not only the expression of the essential replication gene trfA but also its own expression and that of the kilA operon. It has previously been proposed that korA also encodes a positive activator of transcription of the korC gene, which may act as a transcriptional antiterminator. Here we show that the action of korA in relation to korC can be explained entirely through the korA protein's property as a transcriptional repressor. The limited ability of the previously cloned korC gene to suppress kilC on its own is shown to be due to the fact that korC in RK2 is transcribed from the bla promoter of Tn1 which was deleted in the original korC clones. We demonstrate that korA is a second repressor along with korC of three operons, one of which encodes kilC, the other two not having been described previously and serving an as yet unknown function. We have designated these operons kcrA, B and C for KorC-regulated. Putative kilC is designated kcrC. The homology between the expression signals of these operons suggests that they have arisen by duplication. This is confirmed in the case of kcrA and B by the existence of considerable homology between the products of the first ORFs in each of these operons.

INTRODUCTION

Analysis of many bacterial plasmids, for example the classical sex factor F, reveals their present genome organization to be the result of many recombinational events (1). Consequently, it is perhaps not surprising to find that many of the different functions they carry apparently exist as separate 'cassettes' even if they are related by a common purpose. In the case of stable inheritance functions of F, segments responsible for either controlled replication (rep, inc, cop) (for a review see 2), or active partitioning to daughter cells (sop) (3) or lethality to plasmid-free segregants (ccd) (4) can apparently be cut out of the plasmid intact on relatively small segments. In contrast, broad host range plasmid RK2 and other members of the IncP family (for a general review see ref. 5) appear to be unique among plasmids so far studied in

detail. First, their replication system, which consists of a cis-acting vegetative replication origin, oriV, and a gene, trfA, whose protein products act in trans to activate oriV, is not confined to a small contiguous segment of the plasmid but is interrupted by antibiotic resistance markers and/or transposable elements (6,7,8). Second, this replication system is coregulated with a series of other plasmid loci (9,10,11,12). The purpose(s) of these additional loci (kilA, B, C and D), which are host lethal or plasmid inhibitory if not regulated by other plasmid loci (korA/D, B and C) (13,14), is not understood but deletion analysis suggests that they may also play a role in the stable maintenance of RK2. To allow this suggestion to be tested systematically we are characterizing the operons which are coregulated with trfA by determining their DNA sequence and defining the factors which regulate their expression.

In this paper we describe studies on the korC and kilC regions of RK2. The regulation of these loci is particularly interesting because it has previously been found that the product of the key regulatory gene korA can play a role in the control of the KilC$^+$ phenotype (11) as summarized in the top of Figure 1. The hypothesis favoured by these authors to explain this was that KorA (the korA gene product) plays a positive role by activating korC expression. This was based on the observation that the requirement for KorA could be overcome in a rho-strain which is defective in transcriptional termination, raising the possibility that KorA allows transcription through a terminator that normally inhibits korC expression. This positive role for KorA would be in contrast to its well-established role as a repressor of trfA, kilA and korA transcription (9,10,15,12). The results presented here indicate that KorA need not play a positive role. We provide evidence that at least three RK2 operons, one of which seems very likely to encode kilC, are repressed by both KorA and KorC, an observation which can explain the role of KorA in suppression of the KilC phenotype, through its action as a transcriptional repressor.

MATERIALS AND METHODS

Bacterial strains and plasmids and growth conditions

E.coli K12 strains HB101 (for genotype see 16), NEM259 (supE, supF, hsdR, met, trpR) and CSH4 (recA, λag, [λ251]::Tn5) were used. Bacterial cultures were grown in L broth (17) or on solid medium made by addition of 1.5% (w/v) agar to L broth (L agar). Antibiotics were added at

Table 1. Plasmids used in this study.

Plasmid	Replication system	Selective marker	Size kb	Reference
pACYC184	p15A	Cm^RTc^R	4.0	19
pMS202	pMB1	Cm^RPn^R	14.9	20
pMS202A8.2	pMB1	Pn^R	7.4	This work
pCT143	pMB1	Pn^R	6.0	21
pCT673	pMB1	Pn^R	4.0	21
pCAS155	pMB1	Pn^R	6.8	18
pSRW101	IncQ	Tc^RSm^R	14.4	22
pSRW102	IncQ	Tc^RSm^R	14.35	22
pCT720	IncQ	Tc^RSm^R	15.5	This work
pRK229	IncP	Tc^RSm^R	24	7
pUB457	IncP	$Pn^RTc^RKm^RHg^R$	65	23
pUB462	IncP	$Pn^RTc^RKm^RHg^R$	65	23
pUB464-6	IncP	$Pn^RTc^RKm^RHg^R$	65	23
pUB471	IncP	$Pn^RTc^RKm^RHg^R$	65	23
pCT691	p15A/IncP	Cm^RPn^R	28	This work

appropriate concentrations as previously described (18). Plasmids used are listed in Table 1.

Plasmid analysis and in vitro DNA manipulation

Small and large scale preparation of plasmid DNA was carried out by the method of Birnboim and Doly (24). Analysis and construction of plasmids as well as other DNA manipulation techniques were carried out essentially as described elsewhere (16).

Tn5 transposon mutagenesis

Strain CSH4 carrying pMS202A8.2 was spread on L agar containing kanamycin sulphate (50μg/ml). When the bacterial lawn was dry 0.1ml of neomycin sulphate solution (25mg/ml) was placed in the centre of the lawn and allowed to soak in. After overnight growth at 37°C the neomycin had caused a circular patch of inhibition but near its edge appeared colonies showing higher levels of neomycin resistance due to the increased copy number of Tn5 on transposition from chromosome to multicopy plasmid. These colonies were subcultured, plasmid DNA isolated and the transposon insertion mapped.

DNA nucleotide sequence analysis

DNA sequencing was performed by the method of Maxam and Gilbert (25) on DNA fragments which had been labelled either at their 5' or 3' ends by standard methods. Computer analysis was carried out using the 'dna' programme from Dr. G. Ware, Department of Biochemistry, University of Bristol.

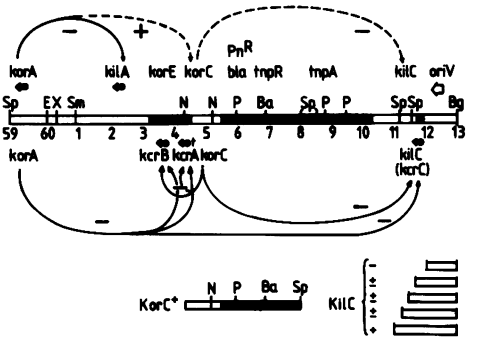

FIG. 1: Physical and genetic map of RK2 from coordinate 58.8 to 13.0kb. Genetic loci are defined in the text. The smallest segment which has been shown clearly to contain korC is indicated, as well as deletions which define the location of kilC. Tn1 DNA is shown as a solid block while the region whose sequence is presented in Figure 3 is shown as a hatched block. Restriction sites are indicated as: BamHI, Ba: BglII, Bg; EcoRI, E; NotI, N; PstI, P; SmaI, Sm; SphI, Sp; XhoI, X. Circuits whose molecular bases are understood are shown as continuous arrows while hypothetical circuits are shown as dashed arrows. Promoters are shown as open circles with direction of transcription indicated by an arrow head. The terminator identified upstream of the kcrA promoter is shown as a 't'. Knowledge prior to this work is summarized above the line while the additional information obtained here is indicated below.

Reverse transcriptase mapping

mRNA was prepared by the method of Sarmientos et al. (26) while reverse transcriptase mapping was carried out as described elsewhere (27).

Catechol 2,3-oxygenase assays

The levels of xylE expression was determined by enzymatic assays using a standard protocol (28) with the exception that protein determinations were carried out using the biuret reaction (29).

RESULTS

Map location of korC

Figure 1 shows a physical and genetic map of the region of RK2 which encodes korA, kilA, korE, korC and kilC. We have analysed the region from coordinates 0 to 5.5kb using a series of deletions originating either at the SmaI site or at the NotI sites of pMS202 (Figure 2). The KorC phenotype of strains carrying these deletion derivatives was determined by transformation with DNA of plasmid pCT691 (Figure 2) which had been cut with BamHI and ligated. If the resident plasmid has a functional korC gene then one should obtain a deletion derivative of pCT691, pCT691.1,

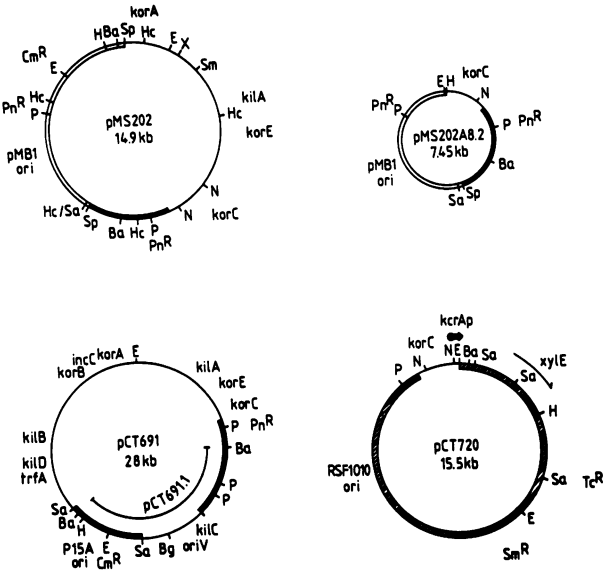

FIG. 2: Key plasmids used in this study. Plasmid pMS202 consists of pBR325 with a single RP4 SphI fragment (kb coordinates 58.8 to 7.7) inserted (20). Plasmid pMS202A8.2 was created from a Bal31 deletion derivative of pMS202 with a HindIII linker inserted, followed by joining of the EcoRI to HindIII korC-containing segment of this plasmid to the small EcoRI segment from pBR322. Plasmid pCT691 was constructed by ligating DNA of plasmids pMS202A8.2 and pSRW102 (22) which had been cut with PstI and EcoRI. Restriction sites are indicated as in Figure 1 with the addition of: HincII, Hc; HindIII, H; SalI, Sa. The sites for the full range of enzymes shown in Figures 1 and 2 are not included on every plasmid but all or none of the sites are shown to avoid confusion.

which has lost the BamHI fragment carrying the korC gene but retains the complete kilC gene. No deletion of this segment should be observed if the resident plasmid does not possess a functional korC gene. Initially we carried out the experiment so that all the strains being transformed also carried the korA gene in trans but during the course of these experiments we found that in our hands korC alone on a high copy number plasmid is sufficient to suppress the KilC$^+$ phenotype. This contrasts with previously reported results in which both korA and korC were required to overcome the KilC$^+$ phenotype (11) a point which is discussed below. However, in agreement with previous observations, we confirmed that korA alone is not sufficient to override the KilC$^+$ phenotype. The results (Figure 1) show that korC is probably encoded between RK2 coordinates 4.4kb

Table 2. Suppression of the KilC$^+$ phenotype by pMS202A8.2 and its derivatives

Resident plasmid	Relative number of pCT691.1 transformants[a]
pMS202A8.2	1.00
pMS202A8.21	0.20[b]
pMS202A8.22	0.37[c]
pMS202A8.23	1.00
pMS202A8.24	0.0016
pMS202A8.25	0.0018

[a]Number of transformants was normalized for bacterial competence by transformation with pCT691. Typically 5×10^4 transformants per μg DNA ($3\text{-}5\times10^2$ colonies per plate) were obtained for pCT691 and pCT691.1 with pMS202A8.2 as resident plasmid.

[b]These colonies were tiny.

[c]These colonies were very small.

and the beginning of Tn*1* at 5.5kb. To define *korC* more closely we isolated Tn*5* insertion derivatives of pMS202A8.2, one of the smallest deletion derivatives which retains a KorC$^+$ phenotype. The results for a key selection of derivatives are shown in Table 2. They show that an insertion 0.5kb counter clockwise from the *Eco*RI site (pMS202A8.23) had no effect on the KorC phenotype whereas insertions 0.4kb clockwise from this *Eco*RI site (pMS202A8.24 and pMS202A8.25) abolished the KorC$^+$ phenotype completely, consistent with the *korC* gene lying to one end of the RK2 backbone DNA in pMS202A8.2. Most interestingly, however, was the finding that insertions 0.2kb on either side of the *Pst*I site in Tn*1* (pMS202A8.21 and pMS202A8.22) resulted in partial inactivation of the KorC$^+$ phenotype. This suggests that while the *korC* structural gene is within the IncP backbone DNA it may depend for expression on the *bla* promoter in Tn*1*, which lies between the *Pst*I and *Bam*HI sites in Tn*1* on pMS202A8.2 (30,31).

Map location of *kilC*

Nucleotide sequence of the region between Tn*1* and *oriV* (to be published elsewhere) has revealed an open reading frame (ORF) preceded by a putative $E\sigma^{70}$ promoter, which seems a likely candidate for the *kilC* gene. Using RP1 derivatives with the transposon Tn*501* inserted throughout the *kilC* region (23) deletions extending clockwise into the *kilC* region were made by *Eco*RI digestion and ligation of these plasmids, thus removing DNA from RK2 coordinate 0 through to the positions shown in Figure 1. By transforming the ligated DNA into strains with and without a high copy number plasmid carrying *korC* we determined which deletions removed the

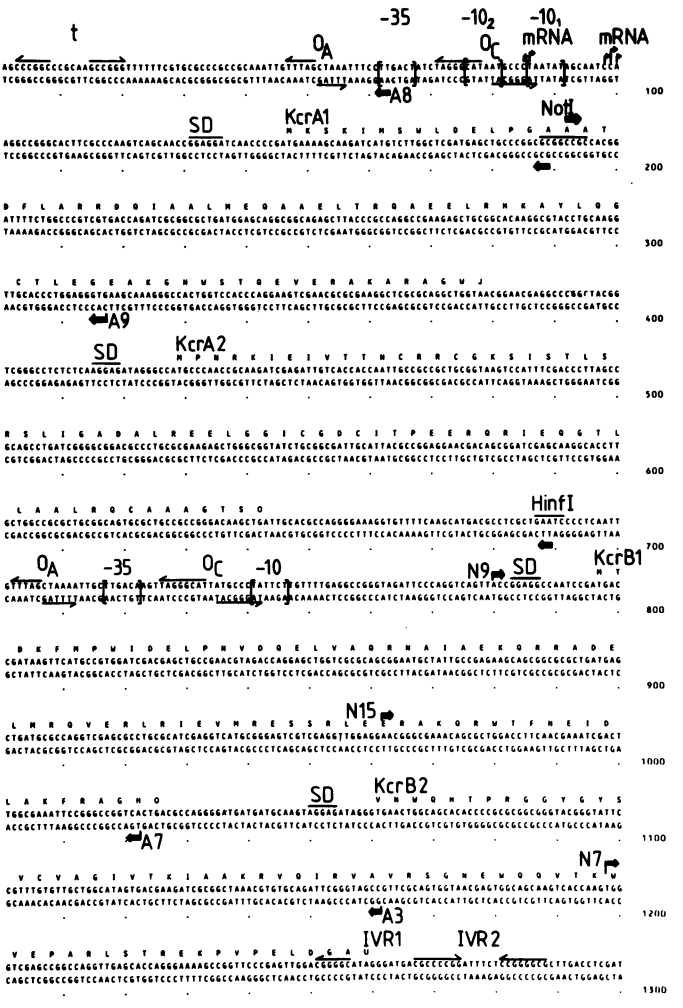

FIG. 3: Nucleotide sequence of RK2 from coordinates 3.3kb to 4.5kb. The sequence was determined from the positions shown with solid arrows, which represent either naturally occurring restriction sites or sites in linkers introduced after Bal31-generated deletion. This provided sequence for both strands over most of the region, including all regions where one strand gave an ambiguous reading. Putative protein coding regions are indicated with the standard single letter amino acid code. Where more than one possible start occurs in an ORF the one with the most reasonable Shine-Dalgarno sequence (SD) is indicated. The -10 and -35 regions of both kcrA and kcrB promoters are indicated. The 5' ends of kcrA mRNA are indicated; the height of the arrow roughly indicates the relative frequency of occurrence. Inverted repeats are indicated by ⟶ ; O_A and O_C represent putative KorA and KorC binding sites, while t refers to a putative rho-independent terminator upstream of kcrA. IV1 and IV1' are alternative inverted repeats downstream of kcrB2.

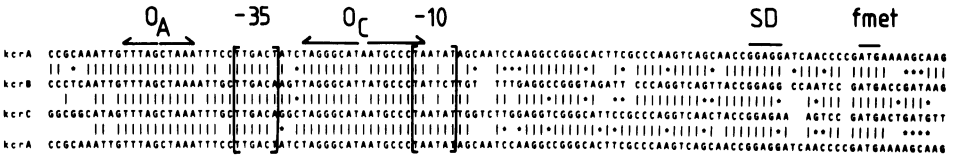

FIG. 4: Comparison of kcrA, B and C expression signals. The KorA binding site (O_A), the -35 and -10 promoter regions and the start codons of the first were aligned and a minimum number of gaps introduced into kcrB and kcrC to maximize homology. Perfect matches are indicated by a line, purine or pyrimidine matches are indicated by a star. SD indicates the proposed Shine-Dalgarno sequence for each gene.

necessity for a KorC$^+$ helper plasmid and which therefore had inactivated or completely removed kilC. Deletions which extended progressively into the putative kilC ORF gradually reduced the requirement for korC but did not completely abolish the requirement until the putative promoter had also been deleted. This gradual loss of KilC$^+$ phenotype suggests that it may be due to a combination of a very strong (kilC) promoter and a host lethal polypeptide product and is consistent with the proposed location and orientation of kilC. It is conceivable that the inhibitory effect of the putative kilC promoter in the absence of a complete kilC ORF is due to transcriptional read through into the korA/korB operon which would in turn increase inhibition of expression of the trfA operon which is essential for plasmid replication. We consider this not very likely since the korA promoter is preceded by an excellent transcriptional terminator (23a). The basis for these effects will be presented in detail when the kilC sequence is published.

Identification of three operons with homologous promoters

Figure 3 shows the nucleotide sequence of RK2 DNA from coordinate 3.1kb to 4.4kb. Analysis of this region, which does not include the korC gene, revealed two segments containing possible E.coli Eσ^{70} promoters (32). These two regions show remarkable homology to each other and to the region located at approximately 11.4 kb on the RK2 map (Figure 4) which we have identified within the nucleotide sequence of the kilC region as likely to contain the kilC promoter (see above). Based on results reported later in this paper we have called these promoters kcrAp, kcrBp and kcrCp for KorC-regulated promoter. Adjacent to the -35 region of each of these promoters is an inverted repeat (O_A) which has previously been implicated in transcriptional repression by KorA (33,15) while overlapping the -10 region is a novel inverted repeat (O_C) which we propose to be the binding site for

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KcrA  MKSKIMSWLQELPGAAATDFLARRDQIAALMEQAAELTRQAEELRHKAYLQGCTLEGEAKGHWSTQEVERAKARAGW
      |  |*| |* |||  * ****| |  ||     | || ||*| ||  *   ** ||  ||**|* *|** ||*|||
KcrB  MTDKFMPWIDELP NVDQELVAQRNAIAEKQRRADELMRQVERLRIEVMRESSRLEERAKQRWTFNEIDLAKFRAGH
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FIG. 5: Alignment of the predicted KcrA1 and KcrB1 polypeptide products. Amino acids are shown by the standard single letter code. A single space has been introduced into KcrB1 to maximize homology. Perfect matches are indicated by a line, matches of similar amino acids (group matches) are shown by a star.

KorC since it is the most obvious novel operator-like feature common to all three promoters.

Immediately upstream of kcrAp is a G+C-rich potential stem loop structure followed by a run of T residues (Figure 3), suggesting the presence of a rho-independent transcriptional terminator (34). A similar structure is not found upstream of kcrB nor kcrC but two inverted repeats are found to follow ORF kcrB2 (see below), one of which is followed by the sequence TTTCT and therefore shows some resemblance to a transcriptional terminator. On the other hand these could simply represent elements which affect the processing and therefore the half life of mRNA produced (35). Assessment of their real significance awaits further analysis.

The kcrA promoter is followed by ORFs coding for potential polypeptides of 78 amino acids, KcrA1, and 71 amino acids, KcrA2, both of which are preceded by adequate translational signals. The kcrB promoter is followed by ORFs coding for potential polypeptides of 77 amino acids, KcrB1 and 73 amino acids, KcrB2, both again preceded by adequate translational signals. A search for homology between these various potential polypeptides revealed that if a single space is inserted into KcrA1 then it can be aligned with KcrB1 to show 30 perfect matches and 18 matches of related amino acids (Figure 5). The putative kilC ORF downstream from the kcrC promoter will be described elsewhere but is not homologous to KcrA1 or KcrB1 (J.P.I., C.A.S. and C.M.T. unpublished).

To check whether or not the putative promoters identified are functional, the 5' end of mRNA transcribed from the kcrA region was determined using the reverse transcriptase primer extension mapping technique. The results are shown in Figure 6. The most important conclusion which can be drawn is that this proposed promoter region does function and therefore, that the initiation of any mRNA originating in this region could be regulated by repressor proteins binding to these operator sites. There are two clusters of bands suggesting that the kcrA promoter contains not one, but two, overlapping promoters. While it is possible

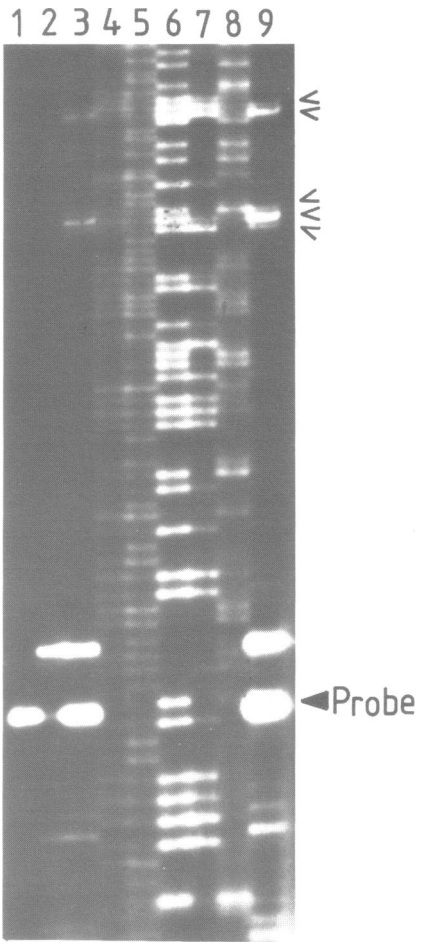

FIG. 6: Reverse transcriptase mapping of the 5' end of kcrA mRNA. The experiment was performed as referenced in Materials and Methods using RNA made from HB101(pCT720). The probe was prepared by kinase labelling of the purified 0.9kb NotI fragment from the korC/kcrA region. This was cut with BglI and the relevant 320bp NotI/BglI fragment purified. Part of this was sequenced to provide the reference tracks 4 to 8 (C, C+T, G+A, G and A C respectively). Some was cut with Sau3AI and hybridized to the purified RNA and then treated with reverse transcriptase and nucleotide triphosphates (tracks 3 and 9). Extended species are indicated by < . Controls consist of DNA probe prior to reverse transcriptase treatment (track 1) and DNA probe treated with reverse transcriptase in the absence of RNA (track 2). In tracks 2 and 3 the band four bases longer than the probe results from filling in of the Sau3AI site in the DNA-DNA hybrid. To determine the base corresponding to the start of transcription it should be remembered that Maxam and Gilbert sequencing destroys the base where the DNA is cleaved so the reverse transcript appears one base longer than it should.

Table 3. Effect of korA and korC repressor genes on the transcription from the trfA and kcr promoters

Coresident plasmid	Relevant loci present in trans	Test promoter trfA (pSRW101)	Test promoter kcrA(pCT720)
pCT673	-	100%[a]	100%[b]
pCT143	korA$^+$	5.8%	5.0%
pMS202A8.2	korC$^+$	111%	4.0%
pMS202	korA$^+$korC$^+$	17%	0%
pMS202A8.21	-	-	66.0%
pMS202A8.22	-	-	66.0%
pMS202A8.23	-	-	4.0%
pMS202A8.24	-	-	85.0%
pMS202A8.25	-	-	99.0%

[a]Absolute level = 29.7 units
[b]Absolute level = 11.5 units

that the faster running bands correspond to a halt signal for reverse transcriptase rather than true 5' termini of mRNA, these bands correspond to the much better Eσ^{70} promoter sequence and so we consider this unlikely. The mRNA from the primary promoter starts at one of three bases while the secondary promoter initiates transcription at one of two possible bases. While the primary promoter shows homology to both the -10 and -35 regions of the Eσ^{70} promoter consensus, the secondary promoter, which was not originally predicted from our homology searches, appears to lack any significant -35 region. Although certain positively regulated promoters of E.coli (36) also lack a -35 region they generally have a conserved TG adjacent to the -10 region (Figure 4). The kcrA promoter has the G but it does not have the T and so the basis for the secondary transcription events remains to be determined.

Regulation of transcription from the kcrA promoter

In order to analyse the transcription from the kcrA promoter a convenient fragment containing it was inserted into promoter probe plasmids which place expression of xylE, coding for catechol 2,3-oxygenase, under the control of the foreign promoter. The level of catechol 2,3-oxygenase is determined by its conversion of catechol to 2-hydroxymuconic semialdehyde whose formation is easily followed by measuring absorbance at 375nm. The structure of the plasmid constructed for this purpose, pCT720, is shown in Figure 2. The plasmid utilizes the IncQ replication system and has a very low copy number. While pCT720 still contains the korC gene in cis the inserted DNA segment runs only to the PstI site in the Tn1 bla gene and therefore lacks the bla promoter. Therefore, the korC in pCT720 should

not be transcribed at a significant level and transcription from kcrAp in pCT720 should be largely constitutive. Table 3 shows that, as predicted, kcrAp is repressed by the presence in trans of either korA or korC. In combination, these repressors completely abolish kcrAp activity. Most importantly it is clear that the ability of korC to cause repression of transcription is not dependent on the presence of korA which would have been expected if korC expression was positively regulated by korA.

The Tn5 insertion derivatives of pMS202A8.2 were tested for their ability to repress transcription from kcrAp (Table 3). As expected the derivatives which abolish the KorC$^+$ phenotype (pMS20A8.24 and pMS202A8.25) do not repress kcrAp. The insertions in the Tn1 bla gene (pMS202A8.21 and pMS202A8.22) retain a small ability to repress kcrAp, consistent with their retention of some KorC$^+$ activity, while the insertion counter clockwise from the EcoRI site in pMS202A8.2 (pMS202A8.23) retains the full ability to repress kcrAp.

To check that the effect of KorC is specific for the kcr promoters we determined its effect on transcription from the trfA promoter using the plasmid pSRW101 (22). We found that it had no effect on the level of xylE expression in this plasmid, while KorA gave the expected approximately 10-fold repression. We therefore conclude that KorC is having a specific effect on the transcription from the kcr promoters.

DISCUSSION

In contrast to a previous report (11) our observations suggest that the korC gene is capable of suppressing the KilC$^+$ phenotype in the absence of korA. This indicates clearly that korA is not required to activate korC and that the level of KorC produced by korC is sufficient to reduce kilC expression to a level which is not lethal to host bacteria. The reason for the discrepancy between previous observations and those reported here may be explained by our finding that korC is transcribed from the Tn1 bla promoter. In the experiments previously reported (11) this promoter was not included in the cloned korC segment, explaining the low korC expression and the inability to suppress kilC. Thus our results provide an alternative explanation for the previous finding that KorA is required to suppress the KilC$^+$ phenotype. Rather than KorA allowing expression of korC it appears that KorA as well as KorC is likely to be a transcriptional repressor of kilC consistent with the role of KorA as a transcriptional repressor in many other operons (9,10,15,12). The previous finding that

the requirement for KorA could be removed in a rho strain (11) may be explained by the rho mutation allowing transcription from a vector promoter, of no normal relevance to korC, to boost korC expression. The expression of korC from a Tn1 promoter suggests that in an ancestral IncP plasmid korC was part of an operon into which Tn1 inserted. Only a subset of IS or Tn insertion events of this sort would be expected to be viable i.e. those which reactivate korC. Interestingly Tn1 differs from Tn3 by a point mutation which creates two new, stronger bla promoters which transcribe out of Tn1 (30,31). The nature of the original korC operon is currently being investigated.

Our results extend the list of operons known to be regulated by korA and emphasize its importance as a central coordinating regulator of the RK2 genes which are likely to be involved in ensuring stable inheritance of this plasmid. These operons form a separate group, being defined by the presence of a KorA operator adjacent to the -35 region and a putative KorC operator overlapping the -10 region. There is clear evidence for a common evolutionary origin for at least the starts of all three kcr operons. Indeed the first ORFs of kcrA and B still show conservation, whereas it appears that a completely different ORF has been fused to the kcrC operon from about codon three of the ORF1. It is intriguing to speculate as to how ancient these duplications are. Do they, for example, pre-date the separation of the IncP group of plasmids into the α and β subgroups (reviewed in ref. 5). Current studies in this laboratory on the sequence of these plasmid regions in the IncPβ plasmid R751 may help to answer this question.

It is still not clear what the role of kilC or any other kil gene is in the biology of IncP plasmids. It is also unclear what role the products of the kcrA and kcrB operons play although recent observations in the laboratory of D. Figurski (J. Kornacki and D. Figurski, personal communication) suggest that kcrB transcription runs across both korE (37) and kilA (12). It should be noted however that there may be a transcriptional terminator downstream of the second kcrB ORF which may modulate this transcription event. In addition, it may be significant that both KcrA2 and KcrB2 show regions of homology to the αhelix-turn-αhelix motif characteristic of many DNA binding proteins (38) as estimated by the systematic method of Dodd and Egan (39). These regions occur at amino acids 9 to 28 and 33 to 52 respectively. Although there appears to be no significant homology between KcrA2 and KcrB2, in

contrast to KcrA1 and KcrB1, it is intriguing that the second ORF in each operon would produce a protein with potential DNA binding properties. While it is not known how KorE acts it is tempting to speculate that one of these two proteins is KorE. We have not yet tested this possiblity. In our functional analysis of these genes the knowledge of the organization of this region can now be used to design further experiments to establish the role both of the proteins encoded by this region and the complex circuits which control their expression.

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REFERENCES

1. Bergquist, P.L., Saadi, S. and Maas, W.K. (1986) Plasmid 15, 19-34.
2. Kline, B.C. (1985) Plasmid 14, 1-16.
3. Mori, H., Kondo, A., Ohshima, A., Ogura, T. and Higara, S. (1986) J. Mol. Biol. 192, 1-15.
4. Ogura, T. and Hiraga, S. (1983) Proc. Natn. Acad. Sci. USA 80, 4784-4788.
5. Thomas, C.M. and Smith, C.A. (1987) Ann. Rev. Microbiol. 41, 77-101.
6. Figurski, D. and Helinski, D.R. (1979) Proc. Natn. Acad. Sci. USA 76, 1648-1652.
7. Thomas, C.M., Meyer, R. and Helinski, D.R. (1980) J. Bacteriol. 141, 213-222.
8. Thomas, C.M. (1981) Plasmid 5, 277-291.
9. Shingler, V. and Thomas, C.M. (1984) Mol. Gen. Genet. 1985, 523-529.
10. Schreiner, H.C., Bechhofer, D.H., Pohlman, R.F., Young, C., Borden, P.A. and Figurski, D.H. (1984) J. Bacteriol. 163, 228-237.
11. Young, C., Bechhofer, D.H. and Figurski, D.H. (1984) J. Bacteriol. 157, 247-252.
12. Young, C., Prince, A.S. and Figurski, D.H. (1985) Proc. Natl. Acad. Sci. USA 82, 7374-7378.
13. Figurski, D.H., Pohlman, R.F., Bechhofer, D.H., Prince, A.S. and Kelton, C.A. (1982) Proc. Natl. Acad. Sci. USA 79, 1935-1939.
14. Smith, C.A. and Thomas, C.M. (1983) Mol. Gen. Genet. 190, 245-254.
15. Theophilus, B.D.M., Cross, M.A., Smith, C.A. and Thomas, C.M. (1985) Nucleic Acids Res. 13, 8129-8142.
16. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor.
17. Kahn, M. Kolter, R., Thomas, C.M., Figurski, D.H., Meyer, R., Remaut, E. and Helinski, D.R. (1979) Methods Enzymol. 68, 268-280.
18. Smith, C.A. and Thomas, C.M. (1984) J. Gen. Microbiol. 130, 1651-1663.

19. Chang, A.C.Y.C. and Cohen, S.N. (1978) J. Bacteriol. 134, 1141-1156.
20. Lanka, E., Lurz, R. and Fürste, J.P. (1983) Plasmid 10, 303-307.
21. Thomas, C.M. (1986) Plasmid 15, 16-29.
22. Pinkney, M., Theophilus, B.D.M., Warne, S.R., Tacon, W.C.A. and Thomas, C.M. (1987) Plasmid 17, 222-232.
23. Grinsted, J., Bennett, P.M., Higginson, S. and Richmond, M.H. (1978) Mol. Gen. Genet. 166, 313-320.
23a. Thomas, C.M. and Smith, C.A. (1986) Nucl. Acids Res. 14, 4453-4469.
24. Birnboim, H.C. and Doly, J. (1979) Nucl. Acids Res. 7, 1513-1523.
25. Maxam, A. and Gilbert, W. (1980) Methods Enzymol. 65, 499-560.
26. Sarmientos, P., Sylvester, J.E., Contente, S. and Cashel, M. (1983) Cell 32, 1337-1346.
27. Debarbouille, M. and Raibaud, O. (1983) J. Bacteriol. 153, 1221-1227.
28. Zukowski, M.M., Gaffney, D.F., Speck, D., Kauffmann, M., Findeli, A., Wisecup, A. and Lecocq, J.-P. (1983) Proc. Natn. Acad. Sci. USA 80, 1101-1105.
29. Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 177, 751-766.
30. Heffron, F., McCarthy, B.J., Ohtsubo, H. and Ohtsubo, E. (1979) Cell 18, 1153-1163.
31. Chen, S-T and Clowes, R.C. (1987) J. Bacteriol. 169, 913-916.
32. Hawley, D.K. and McClure, W. (1983) Nucleic Acids Res. 11, 2237-2255.
33. Smith, C.A., Shingler, V. and Thomas, C.M. (1984) Nucleic Acids Res. 12, 3619-3630.
34. Rosenburg, M. and Court, D. (1979) Ann. Rev. Genet. 13, 319-353.
35. Mott, J.E., Galloway, J.L. and Platt, T. (1985) EMBO J. 4, 1887-1891.
36. Keilty, S. and Rosenberg, M. (1987) J. Biol. Chem. 262, 6389-6395.
37. Young, C., Burlage, R.S. and Figurski, D.H. (1987) J. Bacteriol. 169, 1315-1320.
38. Pabo, C.O. and Sauer, R.T. (1984) Ann. Rev. Genet. 17, 499-525.
39. Dodd, I.B. and Egan, J.B. (1987) J. Mol. Biol. 194, 557-564.