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Temporal Control of Transposition in Tn5

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IS*50*$_R$ is an insertion sequence associated with the transposon Tn*5*. IS*50*$_R$ carries the structural genes for two proteins; one (P1) is the Tn*5* transposase, and the other (P2) is an inhibitor of transposition. These two proteins are translated from two different transcripts, m1 and m2. When bacteriophage λ::IS*50*$_R$ DNA was introduced into a bacterial cell, m1 and m2 were initially at relative levels of about 1 to 2. As time progressed the amount of m1 fell, whereas the amount of m2 continued to increase, until after about 3 h the ratio of m1 to m2 was about 1 to 80. The temporal changes in the levels of these transcripts correlated with temporal changes in P1 and P2 levels and Tn*5* transposition that have been documented in other studies. We measured the stability of the messages and showed that the differences in the levels of m1 and m2 must reflect real differences in the strengths of their promoters and that the changes in transcription kinetics are mediated by the *dam* methylation system of the cell and are not determined by IS*50*$_R$ products. Our results show that the 5′ end of m2 is about twice as stable as that of m1, which raises the possibility that differential message stability does, in part, influence the ratio of inhibitor to transposase.

The bacterial transposon Tn*5* encodes two proteins that not only promote its movement but also regulate that movement (6). P1, a 58-kilodalton protein, is the transposase, whereas P2, a 54-kilodalton protein, is an inhibitor of transposition (10, 16). The inhibitory activity of P2 acts in *trans* on any other copies of Tn*5* in the cell, whereas P1 is primarily a *cis*-acting protein preferentially supporting movement of the element from which it was synthesized. A variety of techniques have shown that the Tn*5* regulatory molecule does not influence the synthesis of the Tn*5* transposition proteins. Thus, no evidence has been obtained supporting regulatory models whereby Tn*5* controls expression of its own genes through *trans*-acting regulatory molecules. This distinguishes Tn*5* from other transposable elements such as Tn*3* (9), Tn*10* (17), Tn*917* (27), and Tn*501* (26), all of which regulate their own movement by regulating expression of their transposases.

For Tn*5* we have proposed that regulation of movement occurs at the level of the transposition reaction after expression of the transposase (12, 16). In our studies, regulation of Tn*5* transposition was measured in cells with fully established Tn*5* sequences compared with control strains, referred to as naive cells (6), in which Tn*5* was absent. Rosetti et al. (23) showed that immediately after Tn*5* enters a naive cell, transposition frequency is initially extremely high but is then shut down within 2 h. In addition, after measuring rates of synthesis of P1 and P2 at various times after infection, these investigators found that more transposase was synthesized in the first 1-h period than during later times. This indicated that a temporal control of Tn*5* transposition existed that acted at the level of gene expression. These results strongly indicated that the earlier conclusion that regulation of Tn*5* is posttranslational (10, 16) was premature and raised the formal possibility that IS*50*$_R$ proteins could indeed regulate IS*50*$_R$ gene expression.

The organization of the genes for P1 and P2 suggests a mechanism by which their expression may be differentially controlled. Tn*5* is a composite transposon that encodes resistance to the antibiotics kanamycin and bleomycin, with the resistance genes flanked by a pair of 1,500-base-pair (bp) insertion elements in inverted orientations. The right repeat, IS*50*$_R$ (Fig. 1), which is a transposable element in its own right (4, 11), codes for P1 and P2 in the same reading frame on an overlapping sequence (24). Recently, Krebs and Reznikoff (18) have shown that this reading frame may be transcribed from either of two promoters. The first promoter directs the synthesis of a long mRNA, m1, that contains the ribosome-binding region and initiating codons for both P1 and P2. The second promoter directs synthesis of a message, m2, which is 30 bp shorter than m1 and encodes only P2. A possible explanation for the results of Rosetti et al. (23) is that early after Tn*5* (or, in the experiments we report here, IS*50*$_R$) enters the bacterial cell, m1 is transcribed in greater abundance than at later times. The current series of experiments began as a test of this hypothesis. In addition, we also examined whether the data of Rosetti et al. (23) showing temporal differences in expression of IS*50*$_R$ proteins contradict our earlier findings that P2 acts at the level of the transposition reaction and not at the level of gene expression.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The *Escherichia coli* and phage strains used in this study are listed in Table 1. The construction of plasmids is described in the references listed in Table 1. pRI161 and pRI162 are plasmids which place transcription under the control of the *tac* promoter. The vector used for introduction of IS*50*$_R$ into a cell was λ430 (λ170::IS*50*$_R$). This phage has the *b*221 deletion of the λ *att int xis* region to prevent integration into the bacterial chromosome and the *red*3 mutation to prevent homologous recombination with the resident prophage.

Enzymes and chemicals. Restriction enzymes and polynucleotide kinase were purchased from New England BioLabs, Inc., Beverly, Mass. Calf intestinal alkaline phosphatase and S1 nuclease were obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. Formamide was from Fluka Chemical Corp., Hauppauge, N.Y. and was deionized

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TABLE 1. Bacterial and phage strains and plasmids used

Strain or plasmid	Relevant characteristic(s)	Source or reference
E. coli		
SY203	F⁻ Δ(*lac pro*)XIII *argE*(Am) Rif^r Nal^r	Laboratory collection
MC1000	F⁻ Δ(*ara leu*) Δ(*lac*)X74 *galU galK strA*	M. Casadaban
SY900	F⁻ *lacI*^{q1}	Laboratory collection
SY1092	SY900 *dam-13*::Tn*9*	Laboratory collection
SY1089	SY203 *dam-13*::Tn*9*	Laboratory collection
Phages		
λ170	λ *b221 red3 imm21 cts*	11
λ430	λ170::IS*50*$_R$	11
λ90	λ*imm* 21	Laboratory collection
Plasmids		
pRI43	pBR322::IS*50*$_R$	11
pRI122	*Xba*I linker at bp 52 in IS*50*$_R$	16
pRI161	pRI43 with *tac* promoter at bp 80 in IS*50*$_R$	13
pRI162	pRI43 with *tac* promoter at bp 90 in IS*50*$_R$	13

before use (8). [γ-^{32}P]ATP was from Amersham Corp., Arlington Heights, Ill.

Infection protocol. A 2-ml portion of an overnight culture of strain SY203(λ90) or SY1092(λ90) was diluted 1:100 in broth (10 g of tryptone, 5 g of yeast extract, 5 g of NaCl, 1 liter of water) supplemented with 0.2% maltose and 10 mM $MgCl_2$. The cells were grown at 37°C with agitation to an optical density at 600 nm of 0.3. The cells were harvested by centrifugation (8,000 × *g* for 10 min) and then suspended in 5 ml of 20 mM $MgCl_2$–20 mM Tris hydrochloride (pH 8.0) at 6 to 8°C. They were then infected at a multiplicity of infection of 4 with freshly prepared phage lysate and kept at 6 to 8°C for 15 min to allow adsorption of phage and synchronization of infection. The cultures were then added to 200 ml of prewarmed (30°C) broth plus 0.2% glucose and grown at 30°C with gentle agitation. Upon the rise in temperature, λ DNA was injected from the adsorbed phage into the cells. Samples (20 ml) were taken at various times for RNA purification (25, 29).

Probe preparation. CsCl-purified pRI122 DNA was cut with *Hpa*I and treated with calf intestinal alkaline phosphatase. After the DNA was cut with *Xba*I, the two resulting DNA fragments were 5′ end labeled with polynucleotide kinase and [γ-^{32}P]ATP. The two fragments were separated on a polyacrylamide gel (8%) and visualized by autoradiography, and the 135-bp probe DNA fragment was eluted from the gel by incubation overnight in buffer X (0.5 M ammonium acetate, 10 mM magnesium acetate, 0.1% sodium dodecyl sulfate, 0.1 mM EDTA) at 42°C. The DNA was concentrated by ethanol precipitation and suspended in 1 mM EDTA–30 mM Tris hydrochloride (pH 8.0).

S1 nuclease protection assay. RNA-DNA hybridizations and S1 digestions were done essentially as previously described (5). For each sample, 100 μg of RNA plus a several-fold molar excess of the probe DNA relative to the homologous RNA were used. Hybridization was done at 50°C for 16 h. The protected DNA fragments were separated on 8.25% polyacrylamide–8.4 M urea gels and visualized by direct autoradiography. Quantitation was by either of two procedures: (i) scanning densitometry of the autoradiographs, using an LKB 2202 Ultrascan laser densitometer (LKB Instruments, Inc., Rockville, Md.) connected to a Hewlett-Packard 3390A integrator (Hewlett-Packard Co., Palo Alto, Calif.); or (ii) liquid scintillation counting of gel slices (after removing urea by soaking in a 10% acetic acid–10% methanol solution and solubilizing the gel with 30% hydrogen peroxide).

RESULTS

The map of IS*50*$_R$ with the relevant markers used in this study is shown in Fig. 1. The two restriction sites shown are those used in making the ^{32}P-labeled probe; the *Xba*I site does not occur in wild-type IS*50*$_R$ but is due to a linker which was inserted at bp 52. In IS*50*$_R$, transcripts m1 and m2 start from positions 66 and 97, respectively (18). To measure the kinetics of mRNA synthesized from IS*50*$_R$, we used the S1 nuclease protection technique. This technique had two advantages for the present study. First, it is very sensitive, allowing detection of as little as one copy of an mRNA per cell. Second, it allowed us to separately quantitate both messages produced by IS*50*$_R$. The two transcripts are each about 1,500 bp long but differ in length by only 30 bp at their 5′ ends. By using a small (35 bp) DNA probe which encompasses the sequence at which the two messages differ and degrading the other 1,365 bp with S1 nuclease, we were able to separate and quantitate the two transcripts.

Amounts of IS*50*$_R$ transcripts vary temporally. The basic experiment used to investigate the kinetics of m1 and m2 transcription was to infect an *E. coli* cell with λ::IS*50*$_R$ (a λ phage containing a copy of IS*50*$_R$). The infected cell was a λ lysogen, which prevented replication of the incoming phage, and the λ::IS*50*$_R$ used was altered so that it could not integrate or recombine into the bacterial chromosome. Thus, the phage was essentially an inert vector for introducing IS*50*$_R$ into the cell. An autoradiograph showing the amounts of the 5′ ends of m1 and m2 produced at various times after infection of strain SY203(λ90) with λ::IS*50*$_R$ is shown in Fig. 2; the amount of m1 declined quickly, whereas the amount of m2 remained high.

The identities of the protected fragments labeled m1 and m2 were confirmed by several means. In experiments for which results are not shown we compared uninfected cells with cells infected with λ only (λ170) and cells infected with

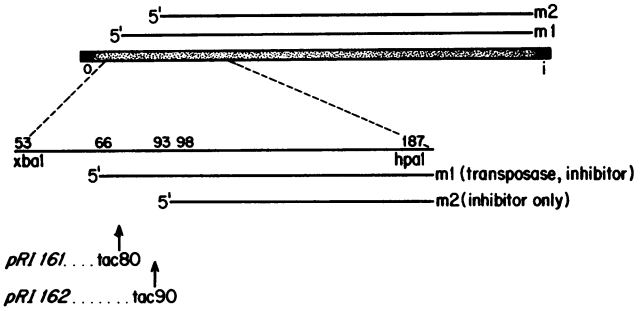

FIG. 1. Structure of IS*50*$_R$. IS*50*$_R$ is a 1,542-bp insertion sequence in which bp 0 is at the outer end (1). The two long transcripts, m1 and m2, begin at bp 66 and 97, respectively. The enlarged section shows the *Xba*I-to-*Hpa*I fragment used to prepare the probe for S1 protection studies. The *Xba*I site is not present in wild-type IS*50*$_R$ but was inserted at bp 52 in pRI122. Also shown is the placement of the *tac* promoter in pRI161 and pRI162. In these two constructs, the native IS*50*$_R$ DNA sequences between the *Xba*I site and the indicated insertion position have been deleted, thus removing the native promoters.

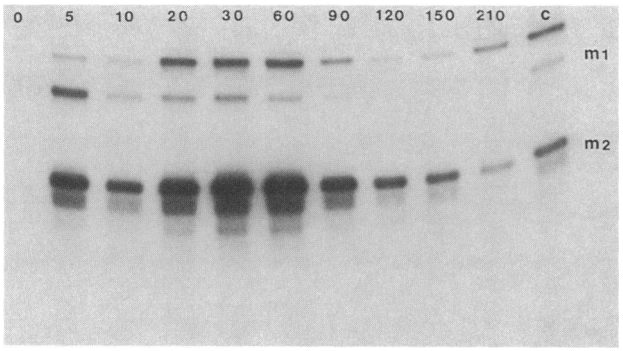

FIG. 2. Autoradiograph of S1-protected fragments. RNA was prepared from strain SY203(λ90) cells infected with λ::IS*50*$_R$ as described in the text. The numbers above the lanes refer to the time (minutes) after infection at which the cells were harvested. Lane c, Transcripts from SY203 carrying pRI43. Identification of the bands labeled m1 and m2 is described in the text. The band above m1 corresponds to reannealed probe DNA.

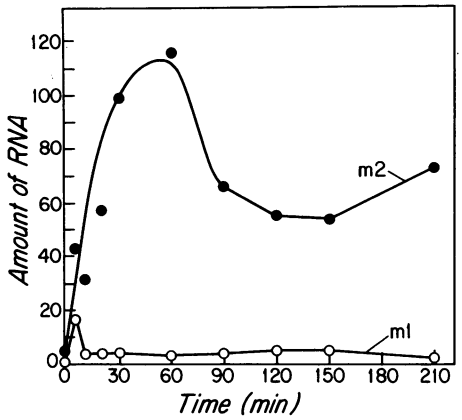

FIG. 3. Kinetics of m1 and m2 synthesized from λ::IS*50*$_R$. The amounts of RNA are from scanning densitometry of autoradiographs. The autoradiograph shown in Fig. 2 was scanned as described in the text, and the relative areas under the densitometry curves for bands m1 and m2 were plotted against time after being normalized for cell growth.

λ::IS*50*$_R$ (λ430, which is otherwise isogenic to λ170) and found that only the λ::IS*50*$_R$-infected cells showed bands m1 and m2. In addition, pRI43, a high-copy-number plasmid containing an intact IS*50*$_R$, was found to synthesize both m1 and m2, whereas pRI122, a mutant of pRI43 that no longer synthesizes transposase (13), synthesized only m2. Moreover, these messages were produced at about 20 to 40 times the amounts seen in λ430 infections, in keeping with the high copy numbers of these plasmids. Lastly, running the fragments m1 and m2 alongside molecular weight markers (pBR322 DNA digested with *Msp*I) showed them to be of the size expected for fragments protecting the 5′ ends of transcripts that begin at the positions indicated and continue past the *Hpa*I site. These results show that the band labeled m1 was found only in transposition-proficient sequences and when P1 (and P2) was synthesized, as was expected from a previous report (18). We conclude that the band labeled m1 reflects the presence of functional message; we have not yet encountered a situation in which the band labeled m1 was synthesized and P1 was not synthesized. Likewise, the presence of the band labeled m2 correlated precisely with the synthesis of P2, and we conclude that m2 monitors the functional message for it.

To quantitate the autoradiogram shown in Fig. 2, film densities were measured with a scanning densitometer (Fig. 3). The densitometric values were normalized to the levels for the initial time point; this was done to compensate for the increase in background RNA due to cell growth (because under our conditions the phage cannot replicate, and therefore the numbers of IS*50*$_R$ copies remain constant). The pattern is consistent with both the transposition kinetics and the levels of P1 and P2 synthesis reported previously (23): the transposase message was initially made at a high level and then declined rapidly to a very low level, whereas the inhibitor message continued to increase. The consequence was that the ratio of m2 to m1 quickly increased at later times, which should have led to a higher ratio of inhibitor to transposase proteins and decreased rates of transposition.

The decrease in m1 is not due to the inhibitor protein. We did two experiments to show that the synthesis of P2 does not cause the result shown in Fig. 3. In the first experiment the levels of transcripts from cells infected with different numbers of IS*50*$_R$ copies were determined. We compared the levels of m1 and m2 produced by IS*50*$_R$ in cells infected with an average of 1, 4, or 8 λ::IS*50*$_R$ copies per cell. The amounts of both transcripts increased linearly with copy number; that is, both the level of transcription per element and the ratio of m2 to m1 remained constant as the number of IS*50*$_R$ copies varied (Table 2).

The second experiment determined the levels of m1 and m2 transcripts from copies of λ::IS*50*$_R$ introduced into cells which already had high levels of P2. High levels of P2 were synthesized from high-copy-number plasmids which have been shown to reduce the transposition frequency of introduced Tn*5* elements several hundredfold (10). To examine this question at the level of transcription, it is necessary to distinguish between the transcripts of the resident elements that are synthesizing P2 and those of the infecting λ::IS*50*$_R$. To this end, we used plasmids pRI161 and pRI162. They contain IS*50*$_R$ with a *tac* promoter fragment (7) inserted at positions 80 and 90, respectively. These plasmids not only eliminate the native promoters for m1 and m2 but replace them with *tac* promoter (Fig. 1). Thus, fully functional P1 and P2 are synthesized from transcripts which differ in length from wild-type m1 and m2. Cells harboring pRI161, pRI162, or pBR322 (containing no IS*50*$_R$ sequences) were infected with λ430, and RNA samples were taken at 30 and 60 min. After the protected fragments were separated on polyacrylamide-urea gels, the gels were sliced into fractions, and the radioactivity in each was counted on a scintillation counter. In this experiment, the resident transcript from

TABLE 2. Levels of m1 and m2 with increasing numbers of IS*50*$_R$ copies

MOI[a]	Time (min)	Amt of:	
		m1	m2
1	30	1.08	11.58
4	30	4.29	31.43
8	30	7.21	63.19
1	90	1.73	13.50
4	90	4.69	25.60
8	90	11.69	67.32

[a] MOI, Multiplicity of infection: the number of phage particles per cell.

pRI161 interfered with quantitation of m1 because of its proximity in the gel and the transcript from pRI162 similarly interfered with quantitation of m2. Therefore, the amounts of m1 produced by the infecting phage were quantitated from the pRI162-containing cells and the amounts of m2 produced were determined from the pRI161-containing cells (Table 3). The presence of the two proteins encoded by $IS50_R$ did not affect the synthesis of m1 and m2 from the infecting λ::$IS50_R$ (Table 3). Therefore, the temporal decrease in m1 synthesis cannot be caused by any kind of feedback repression mechanism involving either P1 or P2.

The temporal decrease in m1 synthesis is due to DNA methylation. A possible cause for the decrease in the level of m1 was suggested by findings that transposition frequencies of Tn5 (22) and levels of P1 (J. C. P. Yin and W. S. Reznikoff, J. Mol. Biol., in press) are increased in *E. coli dam* mutants. The *dam* system methylates adenine in the sequence GATC. Such a methylation site occurs in the −10 region of the m1 promoter (18), and methylation of this site presumably acts to reduce transcription (Yin and Reznikoff, in press). Although the λ::$IS50_R$ used in the infection experiments was grown in *dam*$^+$ cells, phage DNA packaging is very rapid under these conditions and could conceivably outstrip the ability of the *dam* system of the host to find and methylate all GATC sites (19). Thus, at the start of an infection experiment, a proportion of the incoming $IS50_R$ elements could possess unmethylated m1 promoters and transcribe m1 at a high rate. Methylation of these sites by the new host would account for the subsequent decrease in levels of m1. Such a mechanism could also account for the observed transposition kinetics (23).

To test this, we performed parallel infection experiments using strain SY203(λ90) as the *dam*$^+$ recipient strain and either SY1089(λ90) or SY1092(λ90) as the *dam* mutant recipient. The λ::$IS50_R$ used for these experiments was grown on a *dam* mutant so that all entering phage would be completely unmethylated. An autoradiograph of the results of such an infection of SY1089(λ90) is shown in Fig. 4. There are several obvious differences between this autoradiograph and that shown in Fig. 2. The relative levels of m1 and m2 were nearly equal in the absence of methylation; this was due to an increase in the amount of m1 produced since the level of m2 was not significantly different from that obtained with the *dam*$^+$ strain. The averages from two such parallel infection experiments are shown in Fig. 5A. The difference in the kinetics of m1 in the *dam*$^+$ strain and the *dam* mutants is striking. In the absence of host methylation, m1 continued to increase throughout the course of the experiment. The effect of this difference in m1 kinetics on the ratio of inhibitor message to transposase message is shown in Fig. 5B. In *dam*$^+$ cells, the m2/m1 ratio increased throughout the experiment, eventually reaching a value of about 75, whereas in *dam* mutant cells the ratio remained at a little over 2.

TABLE 3. Effect of resident $IS50_R$ on levels of m1 and m2

Transcript (min)	Amt of transcript (cpm)[a]	
	Without resident	With resident
m1 (30)	3,032	2,635
m2 (30)	12,085	14,062
m1 (60)	5,150	3,167
m2 (60)	17,945	18,974

[a] With resident refers to cells containing plasmids which produce P1 and P2. Without resident refers to cells containing pBR322, which do not produce P1 or P2.

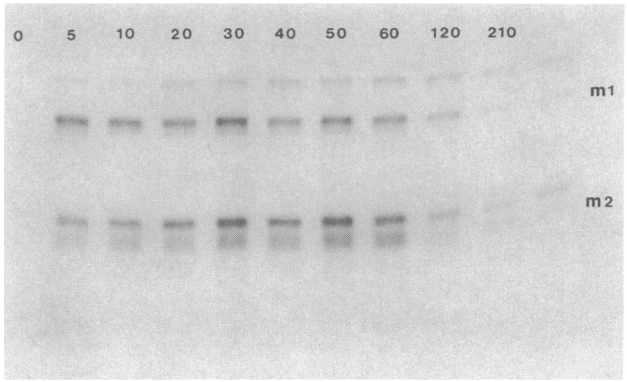

FIG. 4. Autoradiograph of protected fragments from *dam* mutant cells. Phage λ430 grown on the *dam* mutant SY1089 was used to infect strain SY1089(λ90), and RNA was harvested at the times shown.

m2 shows greater stability than m1. The results presented above were interpreted as being due to the differential activities of the two promoters directing the synthesis of m1 and m2. However, in the determinations presented above we measured the total amount of RNA present at each time point. The change in the total amount of an RNA species during any time interval is equal to the amount synthesized minus the amount which decays. Because mRNA in procaryotes is highly unstable, the amount which decays during a given time interval can be significant. We therefore mea-

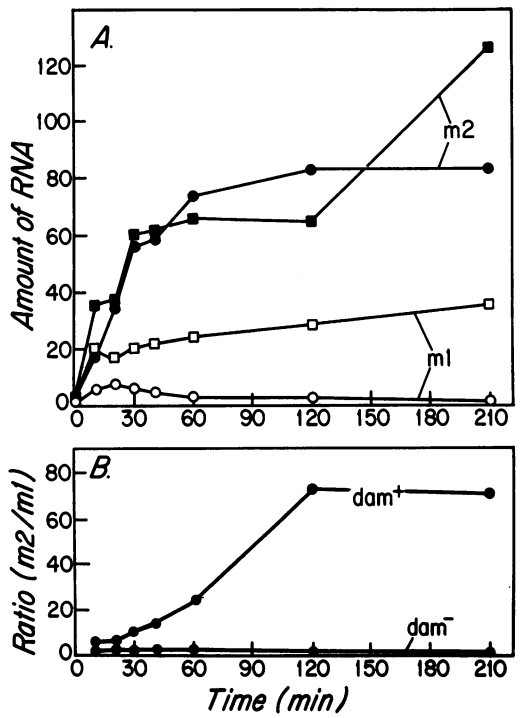

FIG. 5. Kinetics of m1 and m2 in *dam* mutant and *dam*$^+$ strains. (A) Phage λ430 was grown on the *dam* mutant SY1089 and used to infect the *dam*$^+$ strain SY203(λ90) (circles) or the *dam* mutant SY1089(λ90) or SY1092(λ90) (squares). The amounts of m1 are shown by open symbols, and the amounts of m2 are shown by solid symbols; the data are means from two experiments. (B) Averages of the m2/m1 ratios from panel A.

sured the stability of m1 and m2 under a variety of conditions. This was done by adding rifampin to the cultures (which stops new RNA synthesis) and then measuring the amounts of m1 and m2 that remained at various times (Fig. 6).

When rifampin was added to the culture 10 min after infection, the newly synthesized m1 and m2 RNA decayed at similar rates (Fig. 6A; half-lives, 4.6 and 4.3 min, respectively). Thus, we conclude that the decline in m1 observed 5 to 15 min after infection was in fact due to reduced promoter activity.

Nilsson et al. (21) have shown that some *E. coli* mRNA species have different stabilities depending on the growth rate of the cultures. Because in our experiments the cultures grew logarithmically at early times but approached the stationary phase at later times, we also tested mRNA stability 70 min after infection. m2 was significantly more stable ($F = 6.801$; $P < 0.025$) than m1 (half lives, 7.4 versus 4.7 min) at this later time point (Fig. 6B). We observed a similar difference in the relative half-lives when we measured the stability of m1 and m2 synthesized from plasmid pRI43 when rifampin was added to cells that were in the late logarithmic growth phase.

This differential stability must be taken into account in interpreting data like those shown in Fig. 5. At early time points, between 0 and 15 min when the cells are still in log-phase growth, we can attribute differences in the amounts of RNA species to differences in the strengths of their promoters. However, at later times, when the cells are in the stationary phase, the greater stability of m2 is sufficient to account for a twofold excess over m1. Under methylating conditions, the difference in the amounts of m1 and m2 was due to differences both in transcription rates and in the differential stability of the two transcripts, and it is clear that the difference in transcription rate was the more important factor. However, in *dam* mutant cells (Fig. 6) the greater stability of m2 can account for all the difference in the amounts of the two transcripts, allowing us to conclude that the promoters for m1 and m2 were equally efficient in initiating transcription under nonmethylating conditions throughout the course of the experiments.

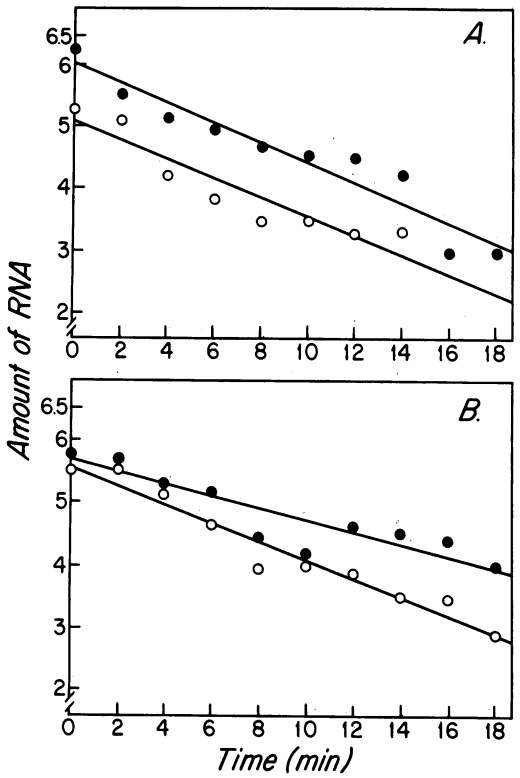

FIG. 6. Stability of m1 and m2. λ430 was used to infect strain SY1092(λ90), and at 10 (A) or 70 (B) min after infection 220 μg of rifampin was added per ml. The addition of rifampin defines the zero time point. The culture was continuously agitated, and cells were harvested for their RNA at the various time points indicated. The amounts of RNA were determined as described in the legend to Fig. 3.

DISCUSSION

After the germinal finding by Biek and Roth of Tn*5* regulation (6), two reports (10, 16) showed that IS*50*$_R$ expresses a regulatory protein that inhibits transposition but does not affect gene expression within IS*50*$_R$. The demonstration by Rosetti et al. (23) of temporal changes in transposase and inhibitor protein synthesis showed that some type of regulated gene expression was occurring. The present study clearly shows that the two conclusions are compatible and that two different regulatory phenomena interplay to account for the regulatory events that Biek and Roth (6) likened to zygotic induction.

Methylation of adenine in the −10 region of the m1 promoter by the *dam* system of the host appears sufficient to explain both the pattern of transcription reported here and the transposition kinetics reported previously (23). Clearly, by reducing transcription from the m1 promoter, methylation greatly increases the amount of inhibitor transcript relative to transposase message. This should be translated into a higher ratio of inhibitor to transposase, eventually reaching a level at which transposition is almost totally blocked. Comparison of the transcription and transposition kinetics shows a difference in timing. Methylation produced a strong decrease in m1 promoter activity at 20 to 30 min after infection, and m1 transcription was almost stopped by 60 min. In contrast, Rosetti et al. (23) demonstrated that transposition continued to occur for about 120 min and then abruptly ceased. Two factors probably account for the difference in this timing of events. The first is simply the lag between transcription, translation, and the action of the proteins (P1 and P2) on their targets. The second is that the ratio of inhibitor to transposase probably has to reach a critical value before an effect is seen. It has been shown (14, 15) that a large amount of P2 relative to P1 is required to significantly decrease transposition and that transposition is insensitive to this ratio over a broad range. Thus, the transposition frequency could be relatively unaffected until the m2/m1 ratio reaches a critical range, after which it is abruptly lowered to almost zero.

Interestingly, the amount of m2 made was not affected by the decreased m1 promoter activity after methylation (Fig. 5A). This implies that the two promoters act independently. We have several lines of evidence to support this conclusion other than seeing no significant difference in m2 levels after infections of *dam*$^+$ and *dam* mutant strains (Fig. 5A). For example, there were no differences in the levels of m2 produced by plasmid pRI43 transformed into the *dam*$^+$ strain SY203 or the *dam* mutants SY1089 and SY1092 (data not shown), although the levels of m1 varied considerably. Comparison of the levels of m2 from pRI43 and pRI122 (in which the m1 promoter is eliminated by an insertion) also showed no difference (data not shown). The fact that these two promoters are only 31 bp from each other indicates that

they overlap. However, activity of the promoter for m1 does not appear to influence the promoter for m2.

The finding that the 5′ ends of m2 show greater stability than those of m1 raises the possibility that the stabilities of their messages differ. There is a formal possibility that the 5′ ends of m1 and m2 (which we assayed by the S1 protection procedure) have greater stability than their functional messages (if this is true, it does not change our interpretation of differential promoter strengths given above). In a few instances (e.g., *ompA*, *bla*, and hybrids thereof) in which the stability of different regions of an mRNA across an entire gene has been examined, degradation appears to occur in concert, as if there is a rate-limiting event that occurs early, after which the message degrades simultaneously. If we generalize from these results to m1 and m2, it implies that the stability determinants for the messages reside at their 5′ ends; this would be consistent with other systems (3).

This conclusion is reinforced by the results of an experiment in which we compared the stabilities of m1 and m2 transcribed from plasmid pRI43 with those of the 5′ ends of the transcripts from pRI161 and pRI162. These latter transcripts differ from m1, m2, and each other only in their 5′ ends, due to the insertion of a *tac* promoter upstream of the translational start of P1. We found that the pRI161 transcript was even less stable than m1 and that the pRI162 transcript was intermediate to m1 and m2 in stability. It should be noted that these stabilities are not simply related to fragment length.

Numerous levels of transposition regulation have been uncovered for bacterial insertion sequences and transposons (28). As has been seen for Tn*10* (11), methylation by the *dam* system of the host greatly reduces transcription of the transposase message, presumably leading eventually to a reduction in the rate of transposition. In the present study we uncovered evidence of another possible level of regulation, with the finding of differential mRNA stability. If this differential stability characterizes full-length transcripts in vivo, it would favor a higher ratio of m2 to m1 in stationary-phase cells and thus a higher ratio of inhibitor to transposase and so provide an additional level of transposition attenuation. This is particularly interesting since *E. coli* is probably not the natural host of Tn*5* (20). Since the *dam* methylation system is rather restricted taxonomically (2), it may well be that the natural host of Tn*5* lacks this system.

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LITERATURE CITED

1. **Auerswald, E.-A., G. Ludwig, and H. Schaller.** 1980. Structural analysis of Tn*5*. Cold Spring Harbor Symp. Quant. Biol. **45**:107–114.
2. **Barbeyron, T., K. Kean, and P. Forterre.** 1984. DNA adenine methylation of GATC sequences appeared recently in the *Escherichia coli* lineage. J. Bacteriol. **160**:586–590.
3. **Belasco, J. G., G. Nilsson, A. von Gabain, and S. N. Cohen.** 1986. The stability of *E. coli* gene transcripts is dependent on determinants localized to specific mRNA segments. Cell **46**:245–251.
4. **Berg, D. E., L. Johnsrud, L. McDivitee, R. Ramabhadran, and B. J. Hirschel.** 1982. Inverted repeats of Tn*5* are transposable elements. Proc. Natl. Acad. Sci. USA **79**:2632–2635.
5. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
6. **Biek, D., and J. R. Roth.** 1980. Regulation of Tn*5* transposition in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. USA **77**: 6047–6051.
7. **deBoer, H. A., L. J. Conostock, and M. Vasser.** 1983. The *tac* promoter: a functional hybrid derived from the *trp* and *lac* promoters. Proc. Natl. Acad. Sci. USA **80**:21–25.
8. **Duesberg, P. H., and P. K. Vogt.** 1973. Gel electrophoresis of avian leukosis and sarcoma viral RNA in formamide: comparison with other viral and cellular RNA species. J. Virol. **12**:594–599.
9. **Heffron, F.** 1983. Tn*3* and its relatives, p. 223–260. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., Orlando, Fla.
10. **Isberg, R. R., A. L. Lazaar, and M. Syvanen.** 1982. Regulation of Tn*5* by the right-repeat proteins: control at the level of the transposition reaction? Cell **30**:883–892.
11. **Isberg, R. R., and M. Syvanen.** 1981. Replicon fusions promoted by the inverted repeats of Tn*5*. The right repeat is an insertion sequence. J. Mol. Biol. **150**:15–32.
12. **Isberg, R. R., and M. Syvanen.** 1982. DNA gyrase is a host factor required for transposition of Tn*5*. Cell **30**:9–18.
13. **Isberg, R. R., and M. Syvanen.** 1985. Compartmentalization of the proteins encoded by IS*50*R. J. Biol. Chem. **260**:3645–3651.
14. **Johnson, R. C., and W. S. Reznikoff.** 1984. Copy number control of Tn*5* transposition. Genetics **107**:9–18.
15. **Johnson, R. C., and W. S. Reznikoff.** 1984. Role of the IS*50*R proteins in the promotion and control of Tn*5* transposition. J. Mol. Biol. **177**:645–661.
16. **Johnson, R. C., J. C. P. Yin, and W. S. Reznikoff.** 1982. Control of Tn*5* transposition in *Escherichia coli* is mediated by protein from the right repeat. Cell **30**:873–882.
17. **Kleckner, N.** 1983. Transposon Tn*10*, p. 261–298. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., Orlando, Fla.
18. **Krebs, M. P., and W. S. Reznikoff.** 1986. Transcriptional and translational initiation sites of IS*50*: control of transposase and inhibitor expression. J. Mol. Biol. **192**:781–791.
19. **Lyons, S. M., and P. F. Schendel.** 1984. Kinetics of methylation in *Escherichia coli* K-12. J. Bacteriol. **159**:421–423.
20. **Mazodier, P., O. Genilloud, E. Giraud, and F. Gasser.** 1986. Expression of Tn*5*-encoded streptomycin resistance in *E. coli*. Mol. Gen. Genet. **204**:404–409.
21. **Nilsson, G., J. G. Belasco, S. N. Cohen, and A. von Gabain.** 1984. Growth-rate dependent regulation of mRNA stability in *Escherichia coli*. Nature (London) **312**:75–77.
22. **Roberts, D., B. C. Hoopes, W. R. McClure, and N. Kleckner.** 1985. IS*10* transposition is regulated by DNA adenine methylation. Cell **43**:117–130.
23. **Rosetti, O. L., R. Altman, and R. Young.** 1984. Kinetics of Tn*5* transposition. Gene **32**:91–98.
24. **Rothstein, S. J., and W. S. Reznikoff.** 1981. The functional differences in the inverted repeats of Tn*5* are caused by a single base pair nonhomology. Cell **23**:191–197.
25. **Scherrer, K., and J. E. Darnell.** 1962. Sedimentation characteristics of rapidly labelled RNA from HeLa cells. Biochem. Biophys. Res. Commun. **7**:486–490.
26. **Schmitt, R., J. Altenbuchner, and J. Grinsted.** 1981. Complementation of transposition functions encoded by transposons Tn*501* and Tn*1721*, p. 359–370. *In* S. Levy, R. Clowes, and E. Koenig (ed.), Molecular biology, pathogenicity, and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
27. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164**: 782–796.
28. **Syvanen, M.** 1984. The evolutionary implications of mobile genetic elements. Annu. Rev. Genet. **18**:271–293.
29. **von Gabain, A., J. G. Belasco, J. L. Schottel, A. C. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80**:653–657.