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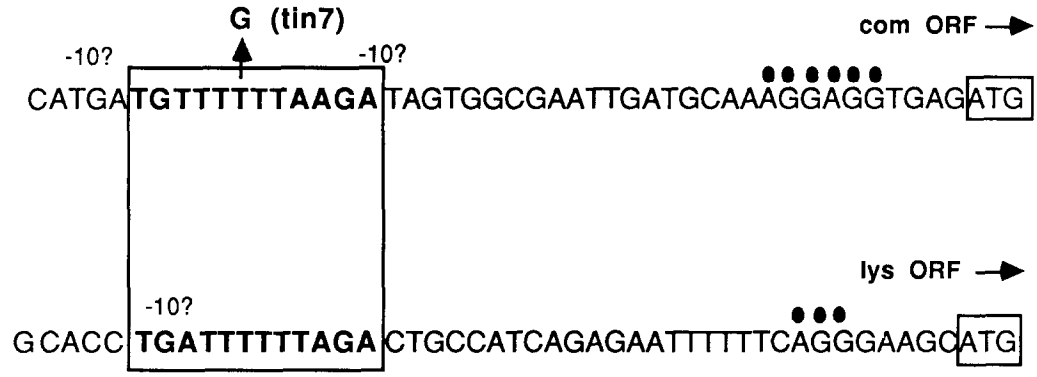
THE PHAGE MU 'LATE' GENE TRANSCRIPTION ACTIVATOR, C, IS A SITE-SPECIFIC DNA BINDING PROTEIN. Valakunja Nagaraja, Gregory Hecht, and Stanley Hattman, University of Rochester, Department of Biology, Rochester, NY, 14627.

The bacteriophage Mu mom gene encodes a DNA modification protein (1). Expression of the mom gene is tightly regulated by at least three known gene products: (i) the host DNA adenine methyltransferase (Dam) is required for mom transcription, presumably by methylating three neighboring GATC sites 5' to the mom promoter; (ii) the Mu C gene product is required for mom transcription, as well as for the "turn on" of all other late genes; and (iii) the Mu com protein appears to be required for translation of the mom mRNA.

We have carried out genetic and biochemical studies to elucidate the role of C in regulating mom expression. A mom-lacZ fusion plasmid was constructed (2) which contains a truncated mom gene fused to the β-galactosidase reading frame. This plasmid synthesizes β-galactosidase activity only in response to those signals which regulate mom; viz. in the presence of C and Com in a dam^+ host. From this plasmid we have isolated and sequenced a spontaneous mutation which results in high level constitutive synthesis of β-galactosidase activity (even in the absence of C protein). This mutation (designated tin7), a single T ---> G substitution in the vicinity of the mom operon promoter -10 site, enables E. coli RNA polymerase to transcribe mom independently of C. The mutation does not improve the poor homology of the -10 sequence; rather, it abolishes the potential static DNA bending (or curving) (3) of a T_6 run (which mutates to T_3GT_2). This suggested the possibility that C protein recognizes a specific DNA sequence, which may have an altered DNA conformation. To test this notion, we first set out to purify the C protein. To this end, we subcloned the C gene into several expression vectors. A variety of constructs were made in which C was placed under control of a regulatable promoter, such as tac, phage λ_{pL} or λ_{pR}. Activation of these promoters generally led to a rapid cessation in cell growth, but most strains did not overproduce the C protein. However, a hybrid protein consisting of Staphylococcus protein A and Mu C was overproduced and purified by affinity chromatography. Proteolytic cleavage releases a peptide containing an intact C plus eight additional amino acids at the N-terminus. This peptide, designated C*, was used in gel retardation experiments to see if we could demonstrate site-specific DNA binding activity. Various plasmids containing the Mu mom promoter region were digested with restriction enzymes and the fragments incubated with C* prior to gel electrophoresis. The results showed that C* specifically retards DNA fragments containing the mom promoter. Therefore, we propose that C is a site-specific DNA binding protein, and that its binding is required for mom transcription.

Because C is required for transcription of other Mu late genes (such as lys), there may be a common transactivation mechanism. Therefore, we constructed a lys-lacZ fusion plasmid to determine if the region 5' to lys was regulated by C. Indeed, the plasmid directed β-galactosidase production only in the presence of C gene function. Moreover, analysis of the nucleotide sequence 5' to the lys gene revealed a site similar to one found in the mom operon promoter region. The two sequences are represented below:



Examination of these sequences suggests the possibility that the 12 base sequence, 5'...TG(A/T) T_5 (T/A) AGA...3', might represent a C recognition/binding site. The following results are consistent with this: i) lys DNA fragments containing the sequence were retarded in the standard mobility shift assay; ii) in contrast, a phage λ DNA fragment containing the sequence with one mismatch, TAA T_5 TAGA, was not retarded.

Experiments are in progress to obtain "DNA footprints" of C protein in the mom and lys promoter regions.

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