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Short Communication

The DNA Sequence Change Resulting from the I^{Q1} Mutation, which Greatly Increases Promoter Strength

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Summary. DNA sequencing shows that the mutational alteration resulting from I^{Q1} is a deletion of 15 base pairs in the *lacI* promoter. The deletion creates a greatly improved −35 homology region, explaining the 50–100-fold increase in *lac* repressor synthesis.

A series of mutations in the *lacI* promoter, which are correlated with varying levels of *lac* repressor synthesis, have aided in elucidating the sequence components required for active transcription. The weak wild-type promoter and the "up" and "down" mutations I^{Q} (Müller-Hill et al. 1968; Calos 1978) and *I-UJ177* (Calos and Miller 1980), respectively, define important features of the sequence. These sequences are best viewed in comparison with a large compilation of information derived from the available promoter sequences (see, for instance, Rosenberg and Court 1979). They confirm and reinforce the conclusion that for prokaryotic promoters specific sequences centered around positions −10 and −35 are recognized by RNA polymerase in the initiation of transcription.

The I^{Q1} mutation results in a level of *lac* repressor 50–100 fold above that of the wild-type (Müller-Hill 1975). We sought to determine the DNA sequence alteration resulting from this mutation. We crossed I^{Q1} onto a small plasmid for sequence analysis by first preparing a double mutant carrying both I^{Q1} and the sequenced mutation *U193* (Farabaugh et al. 1978) on the same F'*lac pro* episome. (*U193* affects the second coding position in the *lacI* gene.) The resulting I^{Q1}, *I-U193* episome was then homogenotized with an I^{+} plasmid, pMCl (Calos 1978), and the resulting I^{-} recombinants were detected as blue colonies on plates containing Xgal (see Miller 1972; and also Farabaugh et al. 1978). The I^{Q1} mutation was assumed to be transferred with *U193* because of the close linkage involved (approximately 50 base pairs).

Plasmid DNA containing the mutations was purified (Tanaka and Weisblum 1975) and digested with *Hin*cII. The 935 base pair fragment bearing the bulk of *lacI* including the promoter extending to position −50 was purified from a 6% acrylamide gel, 5′-end-labelled with ^{32}P and polynucleotide kinase, and recut with *Alu*I. The resulting 200 base pair fragment was isolated from an 8% acrylamide gel and sequenced, all by the methodology of Maxam and Gilbert (1977; 1980). The sequence reveals a 15 base pair deletion contained in the promoter from −29 through −43, as well as the deletion of 2 As in the second codon of *lacI* resulting from *U193*. There were no other changes from wild-type.

The end of the 935 base pair *Hin*cII fragment, which was position −50 of the wild-type promoter, is at position −35 in the I^{Q1} promoter. In order to obtain further upstream promoter sequences, it was necessary to determine the sequence left of the *Hin*cII site. A comparison of the fragments produced by digestion of wild-type and I^{Q1} plasmid DNA with *Hpa*II reveals that a fragment 80 base pairs long in wild-type plasmid changes in size to 65 base pairs in the plasmid carrying I^{Q1}. Thus, this fragment was hypothesized to cover the *Hin*cII site and carry the full I^{Q1} promoter. I^{Q1}, *U193* plasmid DNA was digested with *Hpa*II, labelled with [α-^{32}P]CTP using DNA polymerase Klenow fragment (Calos and Miller submitted for publication) and the 65 base pair fragment was isolated from an 8% polyacrylamide gel. It was recut with the enzyme *Fnu*DII, which recognizes the sequence CGCG (Liu et al. 1979) at position −21, yielding the 43 base pair fragment which was sequenced. The sequence of the I^{Q1} promoter, extended to −50, is shown in Fig. 1. The sequence to the left of the *Hin*cII cut was independently confirmed by sequencing this region of a *Hae*III 200 base pair wild-type fragment spanning the *Hin*cII site (A. Schmitz, personal communication).

The recognition sequence of *Hin*cII is GTPyPuAC, thus position −36 of the I^{Q1} promoter could have been C or T. From comparison with the Rosenberg-Court (1979) consensus sequence, a T exists at this position in 37 of 46 promoters, making it one of the four most conserved bases in the entire promoter. The wild-type *lacI* promoter contains an unfavorable G at −36. On the premise that the I^{Q1} promoter is 50–100 fold stronger than wild-type, we predicted and found that this *Hin*cII site has the most favorable sequence GTTGAC. In fact, the −35 region of I^{Q1} contains a six out of six match with the ideal sequence for the six most conserved bases in the −35 region (TTGACA), compared to a two out of six match for the wild-type promoter. Altogether, the 15 base pair deletion has the effect of replacing the poor −35 region of wild-type with a perfect match to the Rosenberg-Court sequence for the six most important base pairs in the −35 region (Fig. 1b). One can readily attribute the increase in quantity of *lac* repressor found in I^{Q1} strains to increased transcription of *I* mRNA resulting from this now favorable promoter.

Four versions of the *lacI* promoter have been sequenced to date. The wild-type promoter spurs only a low level of transcription, which can be explained by its high G-C content and a poor −35 region with little homology to that of other promoters (Calos 1978). The I^{Q} mutation results in a 10-fold higher level of repressor synthesis than wild-type (Müller-Hill et al. 1968) and contains a single base change from wild-type.

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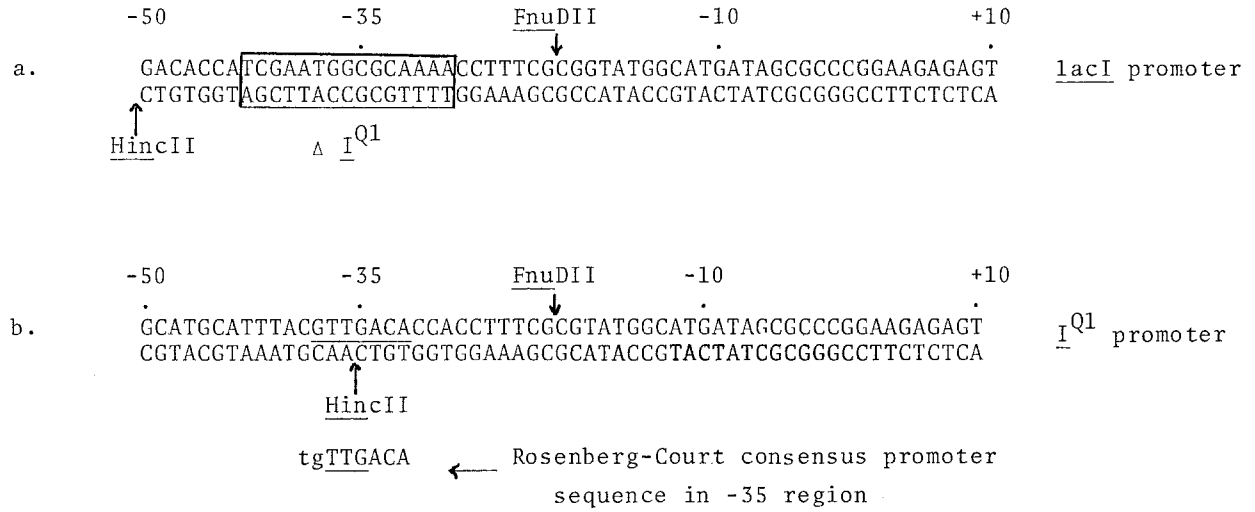

Fig. 1. a Sequence of the wild-type *lacI* promoter showing the location of the 15 base pairs deleted in I^{Q1}. The deletion can be interpreted as extending from position −29 through position −43, or from position −30 through position −44. The locations of the *Hin*cII and *Fnu*DII cuts are also indicated. **b** Sequence of the I^{Q1} promoter extending to −50. The positions of the *Hin*cII and *Fnu*DII cuts are shown. The seven bases homologous to the Rosenberg-Court (1979) consensus promoter sequence in the −35 region are underlined. The consensus sequence is shown below. The more important bases are in capital letters, and the most significant three are underlined

The change from C:G to T:A at position −35 furnishes a critical point of homology and contact to RNA polymerase (Calos 1978). On the other hand, the mutation *UJ177* abolishes promoter activity. It is a deletion of four base pairs destroying the −10 homology region (Calos and Miller 1980).

I^{Q1} was induced from wild-type in a single step by nitrosoguanidine (Müller-Hill 1975). Sequencing shows it to be a deletion of 15 base pairs. Since the G:C to A:T transition is the favored change induced by NG (Coulondre and Miller 1977) it is possible that I^{Q1} is actually a spontaneous mutation picked up in the selection for increased *lacI* expression. The sequences that now form the −35 region in I^{Q1} match the ideal −35 region sequence derived from a comparison of 46 promoters (Rosenberg and Court 1979). Our understanding of prokaryotic promoter sequences has matured to the point of having predictive value. One can only assume that a similar analysis will clarify the nature of the sequence elements of promoters in eukaryotic cells.

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