The Mu gem operon: its role in gene expression, recombination and cell cycle

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Abstract

Two genes, gem\(^A\) and gem\(^B\), belong to the gem operon located in the semi-essential early region of bacteriophage Mu. The product of gem\(^A\) modulates the expression of various host genes, including cell division and DNA replication genes. In addition, GemA is also responsible for decreasing host DNA gyrase activity and for DNA relaxation. The product of gem\(^B\) is involved in Mu late gene transcriptional transactivation. Phage mutants such as Mu gem\(^2\)ts have strong effects on the bacterial host: i) infected bacteria become unable to grow in minimal synthetic medium and behave phenotypically as rel\(^A\) muta ts; ii) survivors of the infection are re-programmed in their cell cycles, with synchronous cell divisions, cyclical waves of DNA relaxation and recoiling and; iii) Mu gem\(^2\)ts prophages excise precisely their DNA from the initial integration site and re-integrate in other non-randomly distributed sites. Neither the phage transposase nor the host RecA protein are implicated in this process.

Introduction

The double nature of Mu, both a temperate bacteriophage and a transposon, makes this genetic element particularly well suited for studies of biological topics such as illegitimate recombination and control of gene expression (for reviews, see Howe, 1987 and Pato, 1989) and gene manipulation experiments (Van gijsegem, Toussaint & Casadaban, 1987). From recent results it also appears that Mu may be a valuable tool in the study of the bacterial cycle (Paolozzi et al., 1989).

Immediately after infection of Escherichia coli K12, Mu DNA integrates at sites apparently randomly distributed along the bacterial chromosome or in other replicons which may be present in the cell (Bukhari & Zipser, 1972, and for review see Harshey, 1987). This integrated Mu DNA is then directed to two possible destinies. In 99.9% of cases it follows the lytic pathway and is replicated through successive cycles of transposition (Ljungquist & Bukhari, 1977; Pato & Waggoner, 1981). At the end of this cycle, the Mu DNA is packaged and new phage particles are liberated by cell lysis. In the remaining 0.1% of cases, the integrated DNA is reduced to prophage and the expression of its repressor gene, \(c\), renders the bacterial lysogen immune to Mu superinfection. This lysogenic state is characterized by the following properties: (i) the target gene is inactivated by the insertion of Mu DNA (Taylor, 1963); (ii) the mutation thus produced is stable due to the rarity of the excision of the insert (< \(1 \times 10^{-10}\)) (Taylor, 1963; Bukhari & Taylor, 1971); (iii) in the progeny of the lysogen, the programmed inversion of a segment of the Mu genome (G-loop) leads to the formation of two classes of bacteria which liberate phage with different adsorption specificities (for review, see Koch et al., 1987); and finally (iv) the expression of the gem operon of the prophage is responsible for a lysogenic conversion involving a complex regulatory network. In addition, infection of sensitive bacteria with some gem\(^-\) mutants induces a reprogramming of the host cell cycle (for review, see Paolozzi & Ghelardini, 1992).

Cellular responses induced by the gem operon in Mu lysogenic bacteria

The first reported manifestation of the activity of the gem operon was the observation that the presence of Mu as a prophage led to the suppression of thermosen-
sensitive mutations in various host genes, including some genes involved in cell division and in DNA replication (Ghelardini, Paolozzi & Liébart, 1980; Bianchi et al., 1990).

This suppression of thermosensitive mutations is coupled with an increased rate of transcription of the corresponding gene, with a decrease of the host DNA gyrase activity and with a global reduction of the degree of supercoiling of the bacterial DNA. A model proposed by Ghelardini et al. (1989) correlates these facts and hypothesises an interaction between Gem and DNA gyrase which would result in a decrease of gyrase activity and hence cause DNA relaxation. Since the expression of many bacterial genes is controlled by the supercoiling of the respective promoter (for review, see Drlica, 1984), such DNA relaxation should modify the transcription of these genes. According to the model, the increase in the rate of transcription of a thermosensitive gene would increase the leakiness by raising the rate of synthesis of the protein coded by the mutated gene, and hence also of that fraction of the protein statistically not thermally denatured. This increased amount of active protein would be sufficient to confer the thermoresistant phenotype. A particularly well documented case is that of the gene which codes for the DNA ligase in E. coli K12 with ts mutations. In agreement with the model, it has been observed that it is possible to suppress the thermosensitive phenotype of these bacteria by compensatory mutations in the gyrB gene which result in DNA relaxation (Liébart et al., 1989). This model is the basis for the discovery that the suppression of the mutated phenotypes is actually only one aspect of a wider response of the bacteria to infection by this phage. Profound biosynthetic modifications in the bacterial lysogens are, in fact, induced by the Gem activity as shown by gel electrophoresis of proteins extracted from lysogens for Mu wild type, from lysogens for the mutant Mu gem3, which behaves as an hyperproducer for Gem, and from the parental isogenic non-lysogenic strain. The electrophoretic patterns, in fact, show the differential presence, or altered abundance, of various groups of proteins (Butler et al., 1991). In addition, these metabolic changes may be emphasized in different culture conditions, as, for example, in minimal synthetic medium.

Despite the rich spectrum of responses of the bacterial cell, the role of the gem operon in the biology of the infected cell and in the development of the phage is still not well defined.

The Mu gem operon: a role in host and phage gene transcription

The gem operon is localized in the semi-essential early region of the bacteriophage Mu, between 8.2 and 9.1 Kb from the left end of the phage genome consists of two genes, gemA and gemB (Paolozzi & Ghelardini, 1992).

The product of the gemA gene is responsible for the suppression of the host lig7ts mutation and for the effects on DNA supercoiling (Ghelardini, unpublished results). The mechanism of action of GemA on DNA supercoiling is still not known and no topoisomerase activity has been found in extracts of bacterial cells infected with bacteriophage Mu (Ghelardini, Pedrini & Paolozzi, 1982).

The behaviour of mutants in gemB has permitted us to assign a role for this gene. By transcription experiments it has been shown that, with these mutants, there is a delay in the synthesis of mRNA for the late genes of the phage and a reduction in the expression of the C gene whose product positively regulates late genes. The Mu C gene appears, therefore, to be one of the primary targets for the action of GemB (Giusti et al., 1990; Mathee & Howe, 1990).

The mechanism by which GemB activates the C gene is not known and the hypothesis that GemB may be a sigma factor of the RNA polymerase has not been confirmed (Mathee & Howe, 1993).

The Mu gem2ts mutant

Extensive properties of the gem operon have been discovered from the behaviour of the thermosensitive mutant gem2ts. Although this mutant is able to grow at 30°C and does not form plaques at 42°C, some of the effects of the mutation are already seen at temperatures permissive for growth (reduced lysogenization frequency and instability of the prophage). Infection at 42°C with this phage is strongly lethal for the host (Buu et al., 1987); amongst survivors, one class called hic shows mutations in the gyrB gene (Ghelardini et al., 1984). This result is the basis for the hypothesis that there is an interaction between the products of the gem operon and the bacterial, gyrase which could justify the reduction of DNA gyrase activity in Mu lysogens (Ghelardini et al., 1984; 1989).

The gem2ts mutation has pleiotropic effects both on the phage itself and on the host. Here we examine the aspects of the bacterial response which have permitted