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Modelling the stability of Stx lysogens

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Abstract

Shiga-toxin-converting bacteriophages (Stx phages) are temperate phages of *Escherichia coli*, and can cause severe human disease. The spread of shiga toxins by Stx phages is directly linked to lysogen stability because toxins are only synthesized and released once the lytic cycle is initiated. Lysogens of Stx phages are known to be less stable than those of the related lambda phage; this is often described in terms of a 'hair-trigger' molecular switch from lysogeny to lysis. We have developed a mathematical model to examine whether known differences in operator regions and binding affinities between Stx phages and lambda phage can account for the lower stability of Stx lysogens. The Stx phage 933W has only two binding sites in its left operator region (compared to three in phage lambda), but this has a minimal effect on 933W lysogen stability. However, the relatively weak binding affinity between repressor molecules and the second binding site in the right operator is found to significantly reduce the stability of its lysogens, and may account for the hair-trigger nature of the switch. Reduced lysogen stability can lead to increased frequency of genetic recombination in bacterial genomes. The development of the mathematical model has considerable utility in understanding the behaviour and evolution of the molecular switch, with implications for phage-related diseases.

Keywords: Bacteriophage; Shiga toxin; Lysogeny; Induction

1. Introduction

Strains of shiga-toxin-producing *Escherichia coli* (STEC) cause diarrhoea, haemorrhagic colitis, and sometimes haemolytic–uraemic syndrome (HUS) in humans (Karmali et al., 1983; Riley et al., 1983) which can be fatal. For example, large outbreaks of STEC O157:H7 infection occurred in primary schools in Japan in 1996 and 1998, affecting over 6000 children and resulting in two deaths from HUS (Watarai et al., 1998).

The major virulence factors of STEC are shiga toxin 1 and shiga toxin 2. The ability of *E. coli* to produce these toxins is conferred by lambdoid bacteriophages known as shiga-toxin-converting bacteriophages (Stx phages). The term 'lambdoid' signifies that these phages share a similar genome structure and life history with the extensively researched phage lambda (Ptashne, 2004). Like all lambdoid phages, Stx phages are temperate—following adsorption to an *E. coli* cell, they are capable of both lytic and lysogenic reproduction. During lytic reproduction, multiple copies of the infecting virus are constructed, and then released—together with toxin molecules—through bacterial lysis (cell burst). Lysogenic reproduction is the mechanism by which the phage DNA (or prophage) becomes incorporated into the bacterial genome, and is then replicated passively during the *E. coli* replication cycle. A lysogen may later enter the lytic cycle through a process known as 'induction'.

In lambdoid phages, a molecular switching mechanism governs the selection of either the lytic or lysogenic pathway, and also determines the rate at which lysogens undergo induction. Lambda prophages are generally very stable, with an intrinsic induction rate of the order of 10^{-7} per cell per generation (Aurell et al., 2002; Little et al., 1999). However, when the survival of the bacterial host is threatened by adverse environmental conditions, such as

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starvation or exposure to ultra-violet light, the rate of lambda prophage induction is known to increase (Ptashne, 2004). It has been shown that the lysogens of the Stx phages 933W and H19B induce more readily than lambdoid phages which do not encode Stx toxin, with intrinsic induction rates of 1.4×10^{-4} and 5×10^{-5}, respectively (Livny and Friedman, 2004). Such Stx lysogens will induce at a lower dosage of 'inducer' (e.g. UV light) compared with lambda lysogens, and this phenomenon is unlikely to be directly caused by the presence of the Stx toxin genes themselves since they are not involved in the switching mechanism. Therefore, 933W and H19B have been described as having 'hair-trigger' switches (Livny and Friedman, 2004), a phrase used to convey the order of magnitude difference in induction sensitivity between these lambdoid Stx phages and the reference bacteriophage lambda.

In Stx infections, shiga toxins are only released when the bacterial cell is lysed, either following the initial infection event or following induction of a lysogen. Thus, the factors regulating the lysis–lysogeny switch play an important role in the regulation of shiga-toxin production and release (Tyler et al., 2004). Using an approach based on the methods of Ackers et al. (1982) and Shea and Ackers (1985), Santillan and Mackey (2004) developed a mathematical model which addresses the high level of stability of lambda lysogens. Various differences between the physical components of lambda and Stx switches have been reported in the literature (e.g. Koudelka et al., 2004; Tyler et al., 2004). The aim of this paper is to contribute to the understanding of the sensitivity of the molecular switch by investigating the impacts of known differences in the molecular binding affinities and structure of Stx and lambda phages on switch dynamics and lysogen stability.

2. Modelling the switch

In lambda lysogens, the concentrations of two regulatory proteins, CI and Cro, determine whether the lysogenic state is maintained or the lytic cycle is initiated. The protein CI represses induction and lysis, as described below, and is therefore referred to as 'the repressor'. The CI and Cro proteins regulate the expression of two genes, *cI* and *cro*, in a feedback mechanism determined by the structure of the genome region associated with the switch (Fig. 1). The O_R region of the genome is situated between the genes *cI* and *cro*, which code for CI and Cro, respectively, and contains the three binding sites O_R1, O_R2, and O_R3. Molecules of CI and Cro in their dimerized form (denoted by CI_2 and Cro_2) bind to these sites and in doing so regulate the expression of the two genes. If a CI_2 molecule is bound to O_R1 or O_R2 then an RNA polymerase (*RNAP*) molecule cannot bind to the *cro* promoter P_R, and so transcription of *cro* is blocked. Similarly, if a CI_2 or Cro_2 molecule is bound to O_R3 then transcription of *cI* is blocked.

The typical molecular configuration at the right operator (see Fig. 1) in a stable lambda lysogen is that O_R1 and O_R2 are both occupied by CI_2 repressor molecules, while O_R3 is unbound (Ptashne, 2004). In this configuration, transcription of *cro* is 'off' but transcription of *cI* is 'on'. The bound repressor molecules continually dissociate from the operator sites, but are replaced by other nearby repressors. The lysogenic state is maintained as long as the concentration of repressor molecules is such that there will always be sufficient nearby repressor molecules to bind to O_R1 and O_R2 when these sites become vacant. If the repressor concentration falls, transcription is initiated by *RNAP* binding at the promoter site P_R during a transient period when O_R1 and O_R2 are unbound. Transcription of *cro* ultimately leads to lysis of the cell and the release of new phage particles.

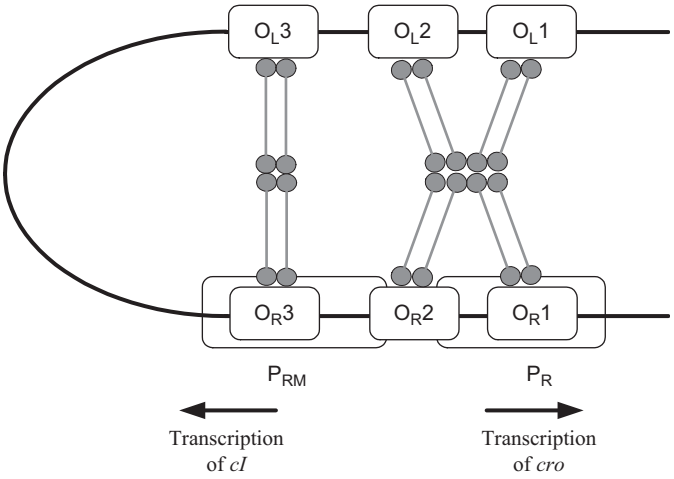

Fig. 1. The region of lambda DNA which comprises the molecular switch between lysis and lysogeny. It includes the left and right operators, which are regions of DNA which can regulate gene transcription. There are three binding sites at the right operator (O_R1, O_R2, and O_R3) and another three binding sites at the left operator (O_L1, O_L2, and O_L3). The promoters for the genes *cI* and *cro* are labelled P_{RM} and P_R, respectively. Repressor molecules are shown bound at all six operator sites. The diagram shows how adjacent repressor molecules interact cooperatively, so as to increase the stability of the molecular configuration. In the configuration shown, transcription of both *cI* and *cro* is blocked. (After Ptashne, 2004).

The left operator region of the lambda genome, O_L, also contains three binding sites: O_L1, O_L2, and O_L3. Experimental evidence due to Dodd et al. (2001) and Ptashne and Gann (2000) indicates that repressor molecules bound at the left and right operators can interact cooperatively to form a stable complex, as illustrated in Fig. 1. It is proposed (Santillan and Mackey, 2004) that this cooperativity contributes to the high degree of stability of lambda lysogens.

2.1. The lambda model

The delay differential equation model of Santillan and Mackey (2004) captures the biochemical processes that govern the lambda molecular switch. It includes the right and left operators, the promoters P_R and P_{RM}, the enzyme *RNAP*, and the proteins CI and Cro in their monomer and