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Restriction–modification systems and bacteriophage invasion: Who wins?

Farida N. Enikeeva [a,*], Konstantin V. Severinov [b,c,e], Mikhail S. Gelfand [a,d,**]

[a] Institute for Information Transmission Problems (the Kharkevich Institute) of RAS, Bolshoi Karetny pereulok, 19, GSP-4, Moscow 127994, Russia
[b] Waksman Institute, Department of Biochemistry and Molecular Biology, Rutgers, The State University of New Jersey, 190 Frelinghuysen Road, Piscataway, New Jersey 08854, USA
[c] Institute of Molecular Genetics of RAS, 2 Kurchatov Sq., Moscow 123182, Russia
[d] Faculty of Bioengineering and Bioinformatics, Moscow State University, Vorobyevy Gory 1-73, Moscow 119992, Russia
[e] Institute of Gene Biology of RAS, 34/5 Vavilova St., Moscow 119334, Russia

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ABSTRACT

The success of a phage that infects a bacterial cell possessing a restriction–modification (R–M) system depends on the activities of the host methyltransferase and restriction endonuclease, and the number of susceptible sites in the phage genome. However, there is no model describing this dependency and linking it to observable parameters such as the fraction of surviving cells under excess phage, or probability of plating at low amount of phages. We model the phage infection of a cell with a R–M system as a pure birth process with a killing state. We calculate the transitional probabilities and the stationary distribution for this process. We generalize the model developed for a single cell to the case of multiple identical cells invaded by a Poisson-distributed number of phages. The R–M enzyme activities are assumed to be constant, time-dependent, or random. The obtained results are used to estimate the ratio of the methyltransferase and endonuclease activities from the observed fraction of surviving cells.

1. Introduction

The phenomenon of restriction–modification (R–M) was discovered in the 1950s during experiments in which different strains of the same bacterial species were infected with bacterial viruses (bacteriophages or phages for short) (Luria and Human, 1952; Bertani and Weigle, 1953). It was observed that while the efficiency of plating (calculated as the proportion of phage particles capable of productively infecting the host bacterium and ultimately leading to plaques, i.e., observable foci of infection on host bacterium lawns) on permissive, non-restricting strains was close to one, efficiency of plating on non-permissive, restricting strains was about five orders of magnitude lower. However, phage progeny that recovered from rare productive infections of restricting hosts were able to plate with equally high efficiency on both restricting and non-restricting strains. Furthermore, the progeny of "modified" phages lost the ability to productively infect the restricting strain after a single passage on the non-restricting strain. Thus, phages recovered from the restricting-strain infections do not contain a heritable change; they are said to be "modified" by the restricting host.

In experiments that ultimately led to the development of molecular cloning and genetic engineering, the molecular basis of R–M phenomena were uncovered. It was shown that restricting hosts encode two enzymatic activities that are absent in non-restricting bacteria (reviewed in Arber, 1978).

The endonuclease molecules can cut DNA at recognition sites. Consequently, they can destroy both the foreign DNA and the genomic DNA itself.

The cell uses methyltransferase to protect its genome from being killed by its own endonuclease, as a methylated site is not recognized by the endonyclease. Moreover, even a hemimethylated site is not recognized and cut, retaining protection of a newly replicated genomic DNA molecule. These sites are then fully methylated by the methyltransferase, and thus the methylated state is stably maintained in multiple rounds of replication.

On the other hand, if the phage DNA becomes methylated in the bacterial cell, it also cannot be cut by the endonuclease. The progeny phages are methylated as well, and further rounds of the infection proceed without interference from the R–M system. This means that the fate of the cell and the phage largely depends on the competition between the methyltransferase and the endonuclease for the sites in the invading phage genome: if all sites in the phage genome are methylated before endonuclease recognizes any one of them, the phage survives, leading to successful infection.

Over the years, many R–M enzyme pairs (R–M systems) have been isolated from diverse bacteria, the search has been mostly driven by the constant need of restriction endonucleases with novel specificities to be used for molecular cloning (REBASE, 2010,

* Principal corresponding author.
** Corresponding author at: Institute for Information Transmission Problems (the Kharkevich Institute) of RAS, Bolshoi Karetny pereulok, 19, GSP-4, Moscow 127994, Russia.
E-mail addresses: enikeeva@iitp.ru (F.N. Enikeeva), severik@waksman.rutgers.edu (K.V. Severinov), gelfand@iitp.ru (M.S. Gelfand).

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http://rebase.neb.com). Cells possessing an R–M system by definition are more resistant to certain phages, obviously an advantageous trait. Analysis of various phages reveals that their genomic DNA contains little or no recognition sequences for restriction endonucleases commonly found in their hosts, or that they use special mechanisms such as heavy methylation of their DNA or specialized antirestriction proteins that bind to and inactivate restriction endonucleases of the host (Tock and Dryden, 2005). Clearly, phages have evolved these mechanisms to avoid the action of the R–M systems of the host.

The protection afforded by the R–M systems against the infecting phage is not absolute, and a cell that is productively infected ends up serving as a source of modified phage progeny that can effectively wipe out the rest of the population. The efficiency of restriction appears to be genetically determined and is both host strain and phage specific. The physiology of the host also appears to play a role. However, the actual mechanisms that lead to and determine the frequency of overcoming the host restriction by phages are unknown. Here, we model the process of phage infection of a bacterial cell harbouring an R–M system. The model makes specific predictions about the efficiency of the phage restriction at varying multiplicity of infection for phage containing different numbers of R–M system recognition sites. We specifically take into account the fluctuations in the amount of restriction endonuclease, methyltransferase, and phage infecting a cell. The results set the stage for discriminative experiments that will allow to confirm or refute the mechanism of phage restriction implicitly assumed in the model and thus increase our understanding of the mechanism of restriction of foreign DNA by cells harbouring R–M systems.

2. Model

We model a culture of bacterial cells that harbors an R–M system and is invaded by a phage. The number of restriction sites N in the phage genome is known, the total number of bacteria in the culture is K, and the total number of phages equals V. The bacterial cells are assumed to be identical up to the effective activities (see below) of restriction endonuclease and methyltransferase denoted by ρ and μ, respectively. The effective activity of an enzyme is the product of the number of molecules of the enzyme and its single-molecule activity. The effective activities ρ and μ can be time-dependent, constant, or randomly depending on the number of enzyme molecules per cell. In the next section we provide details on the concept of effective activity. We assume that the phage is restricted (or modified) before the replication commences. Our first goal is to obtain probabilities of survival or death for a single bacterium, and, simultaneously, the probabilities of productive or abortive infection for a single phage. We start by modelling our system for the case of a single bacterium invaded by a single phage assuming time-dependent activities $\rho(t)$ and $\mu(t)$. Then we generalize our results to the case of a bacterial culture invaded by multiple identical phages. We assume that the number of phages infecting a single cell is Poisson-distributed. The distribution of the number of R and M molecules per cell is assumed to be Poisson and the single-molecule activities are assumed to be constant. We do not consider conversion to the lysogenic state that is modeled, e.g. in Avlund et al. (2009). We also do not model the spatial distribution of susceptible and restricting colonies, or colonies possessing different R–M systems (Gregory et al., 2010).

2.1. Mathematical model

The process of infection of a bacterial cell is modelled by a pure birth process with killing (see, for example, Karlin and Tavaré, 1982; van Doorn and Zeifman, 2005; Coolen-Schrijner et al., 2006 for some general results on this type of processes). We calculate the stationary distribution for the process for a general situation of time-dependent enzyme activities.

Let $R(t)$ be a continuous time Markov process with $N+1$ states $i=0,\ldots,N$ and a so-called "killing state" -1. The system is at the state i if exactly i restriction sites of the phage DNA are methylated. Assume that effective activities of the methyltransferase and the restriction endonuclease in a bacterial cell are time-dependent functions $\mu(t)$ and $\rho(t)$, respectively.

We suppose that at any state i the methyltransferase and the endonuclease select a site to be processed (methylated or cut) with probability $1-i/N$. Thus, at the state 0 the next site will be methylated/cut with the probability 1. In fact, the enzyme molecules select an unmethylated site with probability $1-i/N$ if i sites are already methylated. We assume that the enzyme molecules cannot select the same site simultaneously. We also assume that a methylated site cannot be selected by the methyltransferase again.

If all N sites are methylated, the phage survives and the bacterium dies. In this case the Markov chain hits the absorbing state N. If the restriction endonuclease encounters an unmethylated site, the phage dies and the Markov chain hits the "no-phage state" -1 meaning that the bacterium has survived the phage invasion.

Let $\mu_i(t)=(1-i/N)\mu(t)$, $\rho_i(t)=(1-i/N)\rho(t)$. In fact, $\mu_i(t)$ is the transition rate from the state i to the state $i+1$ at the time t; $\rho_i(t)$ is the transition rate to the state -1 from the state i at the time t. Roughly speaking, $\mu_i(t)h$ is the probability of methylating a site in the phage genome during an infinitely small time interval $h\to 0$ if exactly i sites are methylated at the time t, and $\rho_i(t)h$ is the probability of cutting a site during an infinitely small time interval $h\to 0$ if exactly i sites of the phage are methylated at the time t.

Let $\mathbf{P}_k(t)=\mathbf{P}\{R(t)=k\}$ be the probability that k sites are methylated at the time t. Applying the theory of birth-and-death processes (Karlin and McGregor, 1957; Feller, 1968) we obtain the following system of differential equations

$$\mathbf{P}_0'(t)=-(\mu_0(t)+\rho_0(t))\mathbf{P}_0(t),$$

$$\mathbf{P}_k'(t)=-(\mu_k(t)+\rho_k(t))\mathbf{P}_k(t)+\mu_{k-1}(t)\mathbf{P}_{k-1}(t),\quad k=1,\ldots,N-1 \tag{1}$$

with the equations for the absorbing states

$$\mathbf{P}_N'(t)=\mu_{N-1}(t)\mathbf{P}_{N-1}(t),\quad \mathbf{P}_{-1}'(t)=\sum_{i=0}^{N-1}\rho_i(t)\mathbf{P}_i(t),$$

where the initial conditions are $\mathbf{P}_0(0)=1$, $\mathbf{P}_k(0)=0$, $k\neq 0$.

2.2. Stationary distribution

Solving the system of the differential equations (see Appendix A), we get the stationary distribution of the process $R(t)$,

$$\lim_{t\to\infty}\mathbf{P}_k(t)=\begin{cases}\left(\frac{1}{N}\int_0^\infty \mu(u)G(u)\,du\right)^N, & k=N,\\ 1-\left(\frac{1}{N}\int_0^\infty \mu(u)G(u)\,du\right)^N, & k=-1,\\ 0, & k=0,\ldots,N-1,\end{cases}$$

where $G(u)=\exp\{-(1/N)\int_0^u(\mu(v)+\rho(v))\,dv\}$.