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Modeling bacterial immune systems: Strategies for expression of toxic – but useful – molecules

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A B S T R A C T

Protection of bacterial cells against virus infection requires expression of molecules that are able to destroy the incoming foreign DNA. However, these molecules can also be toxic for the host cell. In both restriction–modification (R–M), and the recently discovered CRISPR/Cas systems, the toxicity is (in part) avoided through rapid transition of the expression of the toxic molecules from "OFF" to "ON" state. In restriction–modification systems the rapid transition is achieved through a large binding cooperativity, and low translation rate of the control protein. On the other hand, CRISPR array expression in CRISPR/Cas systems involves a mechanism where a small decrease of unprocessed RNAs leads to a rapid increase of processed small RNAs. Surprisingly, this rapid amplification crucially depends on fast non-specific degradation of the unprocessed molecules by an unidentified nuclease, rather than on large cooperativity in protein binding. Furthermore, the major control elements that are responsible for fast transition of R–M and CRISPR/Cas systems from "OFF" to "ON" state, are also directly involved in increased stability of the steady states of these systems. We here discuss mechanisms that allow rapid transition of toxic molecules from the unproductive to the productive state in R–M and CRISPR/Cas systems. The main purpose of this discussion is to put relevant theoretical and experimental work in a perspective that points to general similarities in otherwise mechanistically very different bacterial immune systems.

1. Introduction

Bacterial immune systems defend host cell against infection by bacteriophages (bacterial viruses). Two most prominent examples of such systems are restriction–modification systems, and the recently discovered CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR associated sequences) systems. In order to defend the host bacteria against the incoming infection, these systems have to express molecules that can destroy the genome of the incoming virus. While these molecules are evidently useful, they can also be toxic, due to autoimmunity problems. That is, the same mechanism that is responsible for destruction of the foreign DNA, can also, in principle, lead to the destruction of the host genome.

An example of the balance between toxicity and usefulness is provided by the restriction enzyme within a type II restriction modification system (R–M system) (Kobayashi, 2001). Since the restriction enzyme makes cuts in specific DNA sequences, it can, in principle, cut both the DNA of the incoming virus and the host DNA. Destruction of the host DNA is prevented by methylase, which protects the same DNA sequences that are cut by the restriction enzyme. Consequently, unmethylated DNA sequences of the incoming virus will be cut by the restriction enzyme, while the host genome is protected by its methylation.

A quite different type of bacterial immune system is provided by a recently discovered CRISPR/Cas system (Barrangou et al., 2007; Makarova et al., 2006). The system consists of CRISPR array and associated *cas* genes (Al-Attar et al., 2011), and is represented by Fig. 1. CRISPR cassettes consist of identical direct repeats of about 30 bp in length, interspaced with variable spacers of similar length. CRISPR presents an adaptive prokaryotic immune system, which is responsible for defending prokaryotic cell against invaders, so that a match between a CRISPR spacer and an invading phage (bacterial virus) sequence provides immunity to infection. In addition to the match between a CRISPR spacer and the invading phage, CRISPR-associated (*cas*) genes are also required for this immunity. Specifically, one of the Cas proteins (CasE in *Escherichia coli*) is responsible for processing of the long transcripts that correspond to the entire CRISPR locus (called pre-crRNAs), to small interfering RNAs (called crRNAs) (Brouns et al., 2008; Pougach et al., 2010; Pul et al., 2010). Furthermore, a large complex of Cas proteins is, together with crRNAs, responsible for the recognition and inactivation of invading viruses (Al-Attar et al., 2011).

While CRISPR/Cas system has to efficiently recognize foreign DNA, it also has to prevent autoimmunity (Al-Attar et al., 2011). Regarding this, note that crRNAs are complementary to the spacers on chromosomal CRISPR array from which they are transcribed. Furthermore, it is frequently observed that CRISPR spacers are homologous to host chromosomal sequences (Cui et al., 2008). This implies a possibility of spurious recognition, and subsequent destruction, of the host DNA (Al-Attar et al., 2011) by CRISPR/Cas, though the exact process ("antidote") which prevents autoimmunity is still unclear.

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Fig. 1. A scheme of CRISPR/Cas genomic arrangement. The genomic arrangement of different *cas* genes and CRISPR array elements is indicated. R and S within the CRISPR array correspond, respectively to the repeats and spacers; note that the spacer sequences differ from each other, and are labeled by the consecutive numbers (1,2,3,. . .). IGLB and L in figure correspond to the intergenic regions where promoters for, respectively, *cas* genes and CRISPR array are located. Different *cas* genes are labeled by cas1-3 and casABCDE.

From the above discussion, it is evident that bacterial immune systems can employ a quite different mechanisms for expression of toxic molecules. Despite these differences, it may also be useful to think in terms of more general principles that govern expression of toxic molecules inside bacterial cell. For example, expression of a toxic molecule should generally be accompanied by expression of an antidote (e.g. methylation in the case of R–M systems). Furthermore, it seems plausible that generation of a toxic molecule should involve a rapid transition from "OFF" to "ON" state, so that toxic molecules are present in small amounts when they are not needed, but are then rapidly generated upon infection by invasive DNA. Finally, additional, more subtle, principles may also be relevant: e.g. fluctuations of the toxic molecule in its steady state might need to be small, in order to evade that a large fluctuation of the toxic molecule is unmatched by the antidote amount. We will below discuss relevant theoretical and experimental results on bacterial immune systems, with the purpose of pointing to some possible strategies for expression of toxic molecules inside cell.

2. Regulation of R–M systems

We will first discuss regulation of R–M systems. To understand regulation of these systems, it is important to note that they are often mobile and can spread from one bacterial host to the other (Jeltsch and Pingoud, 1996; Kobayashi, 2001). When a R–M system enters a naive bacterial host, the host genome is initially unmethylated, and can consequently be cut by the restriction enzyme. It is, therefore, evident that expression of the restriction enzyme and methylase must be tightly regulated in order to ensure that the bacterial genome is protected by the methylase, before it is cut by the restriction enzyme. This tight regulation is often achieved through a dedicated control protein (C protein) (Tao et al., 1991), and the mechanism which ensures such regulation will be further discussed below.

2.1. Regulation by control protein

A typical gene arrangement in a R–M system is such that the restriction enzyme (R) and the control protein (C) are transcribed together. For definiteness, we will below concentrate on AhdI type II restriction–modification system (which we will further, for simplicity, denote as R–M system). Transcription of both of these proteins is regulated by the control protein C, which binds to the upstream operator sequence (i.e. C protein regulates both its own expression and expression of R gene) (Bart et al., 1999). The main property of transcriptional control by C protein is a large binding cooperativity (McGeehan et al., 2006; Streeter et al., 2004), which is further discussed below.

Basal rate of transcription of C and R genes is very low, due to a low binding affinity of RNA polymerase (RNAP) to the core promoter in the operator sequence (see Fig. 2A) (Bogdanova et al., 2008). In order to activate transcription of these genes, it is necessary to have C proteins. In solution, C protein exists as a monomer, but in order to bind to DNA, it must first form a dimer in solution. In the operator sequence, there are two dimer binding sites. The first dimer binding site is located immediately upstream of the core promoter; binding of C protein to this position leads to transcription activation (see Fig. 2B). The second dimer binding site directly overlaps with the core promoter, so that binding of C protein to this position leads to transcription repression (see Fig. 2C).

Due to a very large binding affinity, as soon as one dimer is bound to DNA, it immediately leads to binding of the second dimer. Due to this, in the absence of RNAP only tetramer can be observed to be bound to DNA (Bogdanova et al., 2008; McGeehan et al., 2006). However, when RNAP is added to the solution, it can displace one of the two C protein dimers in order to form the activation complex (see Fig. 2B). On the other hand, when concentration of C protein is increased, it becomes increasingly entropically favorable to have the other dimer bound to DNA; consequently, at higher C protein concentrations, RNAP is displaced from the core promoter, which leads to formation of the repressor (tetramer complex) (Bogdanova et al., 2008).

In Fig. 2, configurations that correspond to the different arrangements of RNAP and C protein are schematically shown. With each configuration, the appropriate interaction energies are indicated (for the definition of the interaction energies, see the legend of Fig. 2). The weights that correspond to the basal complex (RNAP bound to the promoter) (Fig. 2A), the activator complex (Fig. 2B) and the repressor complex (Fig. 2C) are denoted, respectively, as Z_{RNAP}, Z_{D-RNAP} and Z_T, and given by the following expressions:

$$Z_{RNAP} = K\,[RNAP]\,\exp(-\Delta G_{RNAP}) \tag{1.1}$$

$$Z_{D-RNAP} = K^3\,[C]^2\,[RNAP]\exp(-\Delta G_D - \Delta G_L - \Delta G_{D-RNAP} - \Delta G_{RNAP}) \tag{1.2}$$

$$Z_T = K^4\,[C]^4\exp(-\Delta G_L - \Delta G_R - \Delta G_T - 2\Delta G_D) \tag{1.3}$$

where K is a multiplicative constant (with units of the inverse protein concentration), $[C]$ is concentration of C protein monomers,

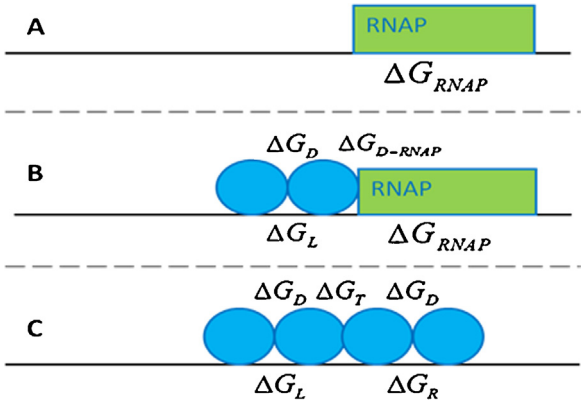

Fig. 2. A scheme of the possible configurations of C protein and RNAP in the promoter region. (A) The basal transcription configuration. (B) The activator configuration. (C) The repressor (tetramer) configuration. ΔG_{RNAP} is the binding energy of RNAP to the promoter; ΔG_D is the dimerization free energy; ΔG_{D-RNAP} is the interaction energy of the dimer with RNAP; ΔG_L and ΔG_R are the interaction energy of C protein dimer with, respectively, the upstream ("left") and the downstream ("right") binding site; ΔG_T is the tetramerization free energy.

while [*RNAP*] is concentration of RNAP. Interaction terms (ΔG's) are defined in the legend of Fig. 2.

These weights in Eqs. (1.1), (1.2) and (1.3) can be used to predict transcription activity of C and R genes as a function of C protein concentration, through widely used Shea-Ackers model (Shea and Ackers, 1985). The Shea-Ackers model assumes that the transcription activity is directly proportional to the equilibrium binding probability of RNAP to the promoter sequence. Consequently, the transcription activity is given by the following expression (Bogdanova et al., 2008):

$$\varphi = \frac{Z_{RNAP} + Z_{D-RNAP}}{1 + Z_{RNAP} + Z_{D-RNAP} + Z_T} = \frac{a + b\,[C]^2}{1 + a + b\,[C]^2 + c\,[C]^4} \tag{1.4}$$

where constants a, b and c can be directly inferred by comparing Eq. (1.4) with, respectively, Eqs. (1.1), (1.2) and (1.3).

In (Bogdanova et al., 2008) we showed that the derived transcription activity (Eq. (1.4)) shows a very good agreement with both the wild type system and systems in which mutations where introduced in one of the two C protein binding sites. Furthermore, changes in the fitted parameters (a, b and c) are consistent with the type of the introduced mutation. We, therefore, conclude that the predicted transcription activity agrees well with the experimental measurements.

One should note from Eq. (1.4) that, for small protein concentrations, the activator complex dominates, and there is a rapid increase of the transcription activity. This rapid increase is a direct consequence of cooperativity in C protein binding. On the other hand, the transcription activity starts to decrease at high protein concentrations, since at high concentrations the repression (tetramer) complex starts to dominate. Another consequence of the cooperativity in C protein binding is a steeper decrease of the transcription activity in the repression regime, which will be relevant for discussion of the stability of the steady state.

2.2. Dynamics of the restriction enzyme generation

We can use the dependence of the transcription activity on C protein concentration in order to model establishment of a R–M system in a naïve bacterial host. To predict this dynamics, we model both the transcript generation and the protein synthesis (for both R and C proteins) as a standard generation/decay processes (Bogdanova et al., 2008). An important consideration in analysis of the system dynamics is that C protein transcripts are leaderless (Laursen et al., 2005), and are consequently poorly translated. Consequently, a significant amount of C protein transcript has to be generated before there is a notable accumulation of C (and consequently R) proteins. Therefore, the poor translation efficiency of C protein transcripts is a mechanism for a delay in the expression of the restriction enzyme, which we will further discuss below.

We can, in a similar way, model the dynamics of the methylation (M) protein synthesis, since it has been shown that the methylase transcription is regulated through a negative feedback loop (Pougach et al., 2010). The dynamics of the restriction enzyme (R) and the methylase (M) synthesis is shown in Fig. 3 (for details see (Pougach et al., 2010)). We see that the restriction enzyme synthesis exhibits a switch-like behavior. Due to this switch-like synthesis, there is initially very little restriction enzyme, so that the host genome can be protected by methylase, before it can be cut by the restriction enzyme. Once the host genome is protected, there is a rapid transition of the restriction enzyme from "OFF" to "ON" state, so that the host bacteria can be protected from the incoming viruses. Therefore, the main property of R–M system is the delay of the toxic protein synthesis (restriction enzyme) with respect to the antidote (methylase), and a fast transition of the toxic molecule from "OFF" to "ON" state.

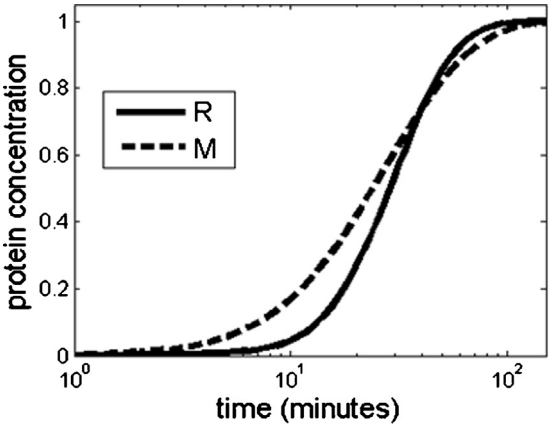

Fig. 3. Dynamics of synthesis of the restriction enzyme (R) and the methylase (M). The full and the dashed curve correspond, respectively, to the concentration of the restriction enzyme and the methylase as a function of time. Zero of time at the horizontal axis corresponds to the entry of R–M in a naive bacterial host. Values on the vertical axes correspond to the protein concentration scaled by the equilibrium value; such scaling allows directly comparing the dynamics of the protein accumulation. The figure is adopted from (Djordjevic et al., 2012).

3. Crispr/cas system regulation

In this section, we will analyze a mechanism for the fast transition from unproductive to productive state of the toxic molecule, which involves control at the level of transcript processing.

3.1. A model of CRISPR transcript processing

It was shown in *E. coli* that CRISPR array and *cas* genes are transcribed from distinct promoters, which are repressed by H-NS (a pleiotropic transcription factor) (Pul et al., 2010). As a consequence, under normal growth conditions, expression of *cas* and (to some extent) CRISPR genes is strongly repressed, so that only a few crRNAs are present in a cell (Pougach et al., 2010; Pul et al., 2010). However, if *cas* genes are overexpressed, or if repression by H-NS is inhibited, there is a large increase of crRNAs, from only few pre-crRNAs (Brouns et al., 2008; Pougach et al., 2010; Westra et al., 2010). Specifically, there is a surprisingly large (two orders of magnitude) increase of crRNAs from only few pre-crRNA molecules, upon *cas* gene overexpression (Pougach et al., 2010). To explain such observations, we will below propose a quantitative model of CRISPR transcript processing, which is based on the experimental results that are summarized below.

As discussed above, it was shown that pre-crRNA is processed to crRNA by CasE. Furthermore, it was shown that pre-crRNAs are also degraded by an unspecified nuclease (Pougach et al., 2010; Pul et al., 2010). Consequently, even in an absence of pre-crRNA processing by CasE, there is a fast decay of pre-crRNA, with a half-life of ~1 min (Pougach et al., 2010); we will further refer to this process, which does not produce pre-crRNA, as the non-specific degradation. Since *cas* and (to a smaller extent) CRISRP promoters are repressed by H-NS (Pul et al., 2010), pre-crRNA and crRNA amounts are low (~10 copies per cell) when the system is not induced (Brouns et al., 2008; Pougach et al., 2010; Westra et al., 2010). When CasE is overexpressed, pre-crRNA amount drops even further (to only a few transcripts per cell), but crRNA amount increases for two orders of magnitude (Pougach et al., 2010). It was also shown that this overexpression of CasE does not influence either pre-crRNA transcription rate, or crRNA stability. Consequently, the surprisingly

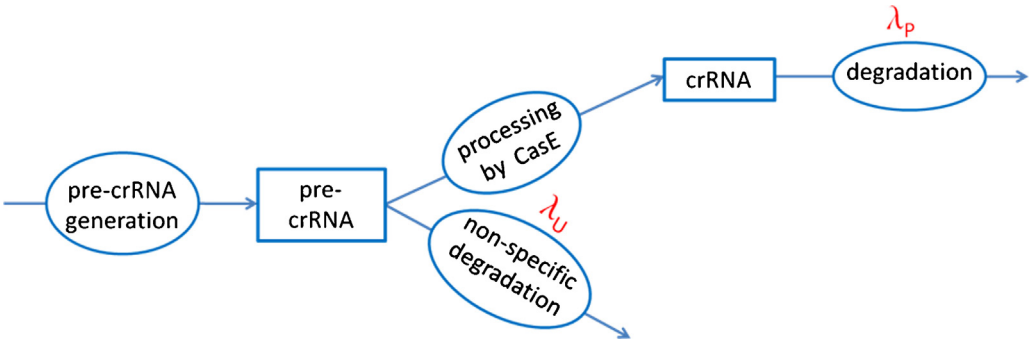

Fig. 4. CRISPR transcript processing scheme. Pre-crRNAs are generated with a certain rate, and are consequently either (non-specifically) degraded with rate λ_u, or are processed to crRNAs by CasE; generated crRNAs are then degraded with rate λ_p.

large increase of crRNAs is only due to an increase of pre-crRNA to crRNA processing rate (Pougach et al., 2010). While it is currently unclear how exactly CRISPR/Cas system is induced under natural conditions (Al-Attar et al., 2011), it was shown that transcription activators – LeuO in particular – can relieve the repression of *cas* promoter by H-NS (Westra et al., 2010). Consequently, a currently prominent model proposes that the induction of the endogenous system involves activation of *cas* and (to a smaller extent) CRISPR promoters, through an abolishment of the H-NS repression (Pul et al., 2010).

These experimental observations lead to the following minimal model of CRISPR transcript processing. The unprocessed transcripts (pre-crRNAs) are transcribed with certain rate, and are consequently either non-specifically degraded with rate λ_u, or processed by CasE to crRNA with rate k; crRNAs are further degraded with rate λ_p. This model is schematically shown in Fig. 4, where the relevant parameters are labeled. Values for these parameters were either directly experimentally determined, or can be inferred from the experimental data by using the model described above (Djordjevic et al., 2012). In particular, there is a very fast decay of pre-crRNAs (λ_u) and a slow decay of crRNAs (λ_p), with the respective decay rates of $\lambda_u = 1\,\text{min}^{-1}$ and $\lambda_p = 1/100\,\text{min}^{-1}$; we will discuss below that this large ratio of the decay rates is crucial for a fast transition of the system from "OFF" to "ON" state.

3.2. Large amplification of crRNA

We will here analyze the surprising observation that a small decrease (less than 10 molecules) of pre-crRNAs leads to a very large increase (more than 1000 molecules) of crRNAs. It is evident that this large "amplification" of crRNAs is directly relevant for the fast transition of the system from "OFF" to "ON" state. We will denote the changes in the number of pre-crRNAs and crRNAs as, respectively, $\Delta[p] = [p]' - [p]$ and $\Delta[u] = [u]' - [u]$. The amounts of unprocessed (pre-crRNA) and processed (crRNA) transcripts are, respectively, labeled as $[u]$ and $[p]$, while the corresponding quantities after the system inductions are labeled by primes. In this subsection, we will focus on the analysis of the steady state of the system, since this regime corresponds to the measurements discussed above.

Detailed kinetic equations that correspond to the model discussed above are provided in (Djordjevic et al., 2012). From these equations it is straightforward to derive the relationship between the changes in the number of pre-crRNAs ($\Delta[u]$) and crRNA ($\Delta[p]$), upon CasE overexpression:

$$\Delta[p] = -\frac{\lambda_u}{\lambda_p}\Delta[u] \tag{1.5}$$

Note that the decrease in the number of unprocessed transcripts (pre-crRNAs) is accompanied by an increase in the number of processed transcripts (crRNAs), which is indicated by the minus sign in the equation above.

From Eq. (1.5) follows that there is a linear relationship between crRNA increase and pre-crRNA decrease; note the large constant of proportionality in this relationship ($\lambda_u/\lambda_p = 100$ – see the previous section). Therefore, Eq. (1.5) shows that the system acts as a strong linear amplifier, where the increase of crRNA is directly proportional to the decrease of pre-crRNA. This strong amplification explains the surprising experimental observations discussed above: note that according to Eq. (1.5), ~10 molecule decrease in pre-crRNA ($\Delta[u] = 10$), leads to a two orders of magnitude larger increase in crRNA ($\Delta[p] = 1000$), as roughly observed in the experiments. The large constant of proportionality ensures that a small number of pre-crRNAs is amplified to a large number of crRNAs. This large amplification is directly relevant for the efficient transition of the system from "OFF" state (with only few crRNA molecules) to "ON" state (with a large number of crRNA molecules).

3.3. Kinetics of crRNA generation

In the previous section, we showed that, in the steady state, the system can generate a very large amount of product (crRNAs), from the substrate (pre-crRNAs) that are consistently kept at low levels. However, the steady state regime may not be directly relevant for CRISPR/Cas function under natural conditions, where the amount of generated crRNAs immediately after induction (e.g. after virus infection) may be more relevant. We, therefore, next discuss kinetics of crRNA accumulation, in order to understand how fast the system can achieve crRNA levels that can protect host bacteria from foreign DNA invasion. While it is hard to experimentally assess how transcripts accumulate with time, this analysis can be readily done through mathematical modeling; to achieve this, we use the model of CRISPR transcript processing that was formulated above.

Since both *cas* and (to a smaller extent) CRISPR promoters are repressed by H-NS, it is widely accepted that the natural system induction involves abolishing this repression (Pul et al., 2010). Consequently, we below consider what happens if transcription of both *cas* genes and CRISPR array is activated. The activation of transcription of *cas* genes and CRISPR array leads to increasing both pre-crRNA to crRNA processing rate and CRISPR transcription rate. Note that the analysis discussed in the previous subsection corresponds only to the increase of pre-crRNA to crRNA processing rate, as is relevant for CasE overexpression experiments in which the transcript numbers were quantified.

In Fig. 5, we show the kinetics of crRNA accumulation for the parameters which are likely close to the natural system induction (Djordjevic et al., 2012). Specifically, experiments show that repression of the *cas* promoter by H-NS is much stronger compared to the repression of the CRISPR array (Pul et al., 2010; Westra et al.,

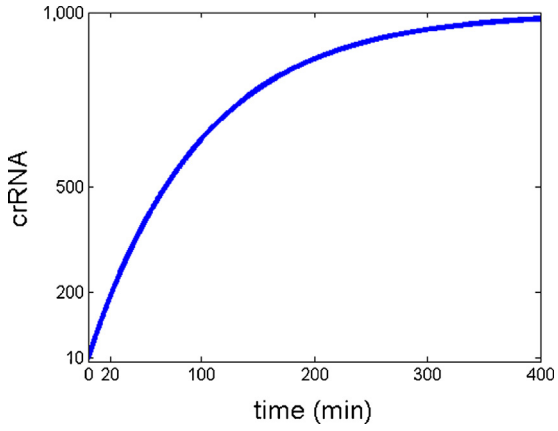

Fig. 5. Kinetics of crRNA accumulation. The figure shows how crRNA changes as a function of time, when pre-crRNA to crRNA processing rate is increased for two orders of magnitude, while CRISPR transcription rate is increased two times. Horizontal axis corresponds to time post-induction, while the vertical axis corresponds to the number of crRNA transcripts.

2010). Consequently, it is likely that when H-NS repression is abolished, transcription of *cas* genes is activated to a much larger extent than transcription of CRISPR array. Furthermore, the increase of the steady state amount of crRNA (approximately two orders of magnitude), which is shown in Fig. 5, is roughly in agreement with the values measured in experiments in which H-NS repression is abolished (Pul et al., 2010; Westra et al., 2010). This provides another argument that the induction parameters used in Fig. 5 are likely close to the conditions relevant for the natural system induction.

Related with the discussion above, Fig. 5 shows that the steady state is reached relatively slowly, i.e. ~300 min after the system induction. On the other hand, the lysis of *E. coli* upon a lytic phage infection is typically complete much before 300 min post-infection; e.g. for the well known T7 and T3 phages, the cell lysis starts at ~20 min post-infection, while the complete shot-off of the host functions occurs much earlier (Kruger and Schroeder, 1981). Therefore, crRNA amounts soon after the system induction (e.g. at ~20 min post-induction), rather than the steady-state crRNA levels, are likely more relevant for the defense against foreign DNA.

From Fig. 5, we see that the transcript amounts at ~20 min. post-induction (~200 transcripts) are indeed significantly lower compared to the steady state crRNA amounts (~1000 transcripts). However, the number of crRNAs at 20 min is still much higher compared to crRNA levels that were experimentally shown to provide a partial protection against bacteriophage infection (~10 crRNA transcripts as per (Pougach et al., 2010)). Therefore, the results strongly suggest that activation of *cas* expression and CRISPR array leads to a rapid accumulation of crRNA, which can provide an effective protection against phage infection. Consequently, induction of CRISPR/Cas system also involves a rapid transition from the unproductive to the productive state of the system.

4. Steady state stabilities

We have analyzed two important bacterial immune systems, which use very different mechanisms to protect a bacterial cell against virus infection. Despite these differences, we have seen that both of these systems exhibit a fast transition from "OFF" to "ON" state. In this subsection we briefly analyze if there are additional, more subtle, principles that determine design of bacterial immune systems. We will below discuss R–M and CRISPR/Cas systems and argue that an increased stability of the steady state may be an example of such principle. Such increased stability of the steady state would prevent large fluctuations of the poison molecule that may be unmatched by the amount of the antidote. We will below briefly discuss mechanisms that allow increased stability of the steady state in R–M and CRISPR/Cas systems.

In R–M systems, we discussed above that large cooperativity in the formation of the repressor (tetramer) complex leads to a steeper decrease of the transcription activity versus C protein concentration. It is straightforward to show that this steeper decrease (e.g. the large cooperativity in C protein binding) leads to a larger stability of the steady state: For example, a direct consequence of this steeper decrease is that a perturbation which increases the steady state C protein amount leads to a larger decrease of the transcription activity; such larger decrease in the amount of generated C protein transcripts leads to a more rapid diminishing of the perturbation. Consequently, the large cooperativity in C protein binding, which is the main property of the regulation of R–M system directly leads to a larger stability of the steady state of the system.

An important control element of CRISPR/Cas system is fast non-specific degradation of pre-crRNA by an unidentified nuclease; as discussed above, this fast processing is a major element that allows fast transition of the system from "OFF" to "ON" state. In addition, it is straightforward to see that this fast non-specific degradation increases stability of the steady state of the system: For example, if there is a perturbation which increases steady-state concentration of pre-crRNA, larger transcript decay will lead to a faster diminishing of this perturbation. Therefore, a major control element of CRISPR/Cas response also directly leads to increased stability of the steady state of the system.

5. Conclusion

The two types of bacterial immune systems that we have analyzed employ a quite different strategies for expression of toxic molecules. In R–M systems, the large cooperativity and the small translation initiation rate of the control protein lead to a switch-like synthesis of the restriction enzyme. As a consequence, the toxic molecule is synthesized with a delay with respect to the antidote (methylase), while the transition from "OFF" to "ON" state happens in a narrow time interval. Once the steady state of the system is reached, its stability is increased by the large binding cooperativity of the control protein.

On the other hand, the fast transition from "OFF" to "ON" state in CRISPR/Cas system is exhibited at the level of the transcript processing, and it crucially depends on the fast non-specific degradation of pre-crRNA by an unidentified nuclease. Consequently, this nuclease is a major control element of CRISPR/Cas response. The large decay rate of pre-crRNAs also increases stability of the steady-state for this system.

Consequently, despite evident mechanistic differences, it may be useful to consider some "unifying" principles that govern expression of the toxic molecules inside cell. Examples of such principles may be a fast transition of the system from "OFF" to "ON" state, or increased stability of the steady-state of the system. Further study of the bacterial immune systems may lead to discovery of more such principles, which may be useful not only for understanding of the endogenous systems, but also for construction of useful synthetic gene circuits.

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