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The function of temporally ordered viral gene expression in the intracellular replication of *Herpes simplex* virus type 1 (HSV-1)

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Contents

The function of temporally ordered viral gene expression in the intracellular replication of Herpes simplex virus type 1 (HSV-1).

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Abstract

In the reproduction of HSV-1, the temporal profile of the viral gene expressions and the molecular mechanisms regulating the expressions are extensively studied. Functional roles of the temporally ordered gene expressions has not yet been clarified. We construct a simple mathematical model for the intracellular replication of HSV-1 to investigate the function of the ordered gene expressions.We obtain the condition for the eexplosionf of the virus from our model. The expression ratio of the early gene to the late gene must be higher than the ratio of the reaction rate of the encapsidation to that of the viral DNA replication for viruses to reproduce successfully. The preceded accumulation of the early gene product prevents the growth arrest. Further, as promoter activity of the early gene becomes higher, the replication speed of virus becomes faster. The structure of early gene promoter that has many binding motif to transcription factor accelerates the replication speed of HSV-1. This structure of the early gene promoter might be selectively maintained by allowing fast growth of the virus. With amino acid limitation, there exist finite optimal ratio of early/late gene promoter activity.

Keywords: HSV-1, immediate early gene, early gene, late gene, gene expression pattern, replication speed

1 Introduction

Herpesviridae comprise a large class of animal viruses, and is important for the public health. Among *Herpes Viridae*, the reproduction of herpes simplex virus type 1 (HSV-1) is the most extensively studied system(Boehmer & Nimonkar, 2003; Stow, 2000). In the replication process of the HSV-1, the temporal profile of viral gene expression is well understood. In the HSV-1 genome the majority of the enzymes required for their own DNA replication is encoded. The mechanism of HSV-1 replication is well studied as a useful general model for eukaryotic DNA replication. In this study, we focus on a function, rather than the mechanism, of temporally ordered expression of viral genes in the replication of HSV-1.

The viral gene products are subdivided into three classes by the timing of their expression (Boehmer & Lehman, 1997). The reproduction of HSV-1 has started when viral particle invades into the host cell through the specific receptor. *Immediate early* genes are first expressed during the reproductive process by VP-16 protein packaged in the viral particle without the synthesis of other viral gene products (Wysocka & Herr, 2003). Shortly after the expression of immediate early gene, *early* genes are expressed, and then *late* genes are expressed. The viral genes are classified not only by the timing of their expression but also by their functions (Nishiyama, 2004). All of the immediate early genes encode the transcription factors which form the transcriptional complex with host transcription factor. The immediate early gene products positively regulate both early and late gene expressions. Early genes generally encodes subunits of DNA polymerase and DNA binding protein functionally associated with viral DNA replication. Whereas, late genes encode the envelope, capsid and tegment protein, i.e., the structural proteins of the viral particle.

The temporal order of HSV-1 gene expression is mainly determined by their promoter activities. Transcriptional activity of the viral genes is regulated by the structures of their promoters (Weir, 2001; Yamamoto *et al.*, 2006). In the immediate early gene promoter, there exist the binding motif, TAATGARAT, where R is represents purine (A or G), to which the transcriptional complex

composed of VP-16 and host transcription factor binds. In addition to TAATGARAT motif, the immediate early gene promoter contains many other binding motifs for binding the cellular transcription factors in the upstream of the TATA box.

The expressions of both early and late genes are regulated by the immediate early gene product such as ICP4 (Kim *et al.*, 2002). The temporally ordered expression of early and late genes is controlled by the difference in the structure of their promoters. Unlike the late gene promoter which has only a few binding motif, both immediate early and early gene promoters contain many binding motifs to which the host transcription factors, such as SP-1, bind. This makes binding affinity of early gene promoter to transcription factor higher than that of late gene promoter. As a result, the early genes are expressed earlier than the late genes.

As mentioned above, the mechanism regulating the temporally ordered expression of early and late genes is well understood. However, the functional role of the temporally ordered viral gene expression in the whole process of viral replication is not yet clarified. We investigate how the temporal pattern of the viral gene expression influences the reproduction of HSV-1 by constructing a mathematical model for the intracellular replication of HSV-1.

2 A mathematical model for HSV-1 replication

The replication cycle of HSV-1 is schematically illustrated in Fig 1. More than 80 genes are encoded on HSV-1 genome. Five, 12 and 56 genes are classified into immediate early, early and late genes, respectively. The genes classified into each group show the common expression pattern. The concentrations of mRNAs and proteins of each group are addressed as one variable in our model. The replication of HSV-1 starts when viral particle invades the nucleus. The immediate early gene mRNA, R_I, is produced by the host transcriptional machinery with the rate γ_I from the viral DNA, D. The immediate early gene product, I, is then translated with translation rate β_I. mRNA and protein of immediate early gene product are degraded with the degradation rate δ_{R_I}

and δ_I, respectively. The chemical reaction equation concerning the immediate early gene is then

$$\begin{aligned} D &\xrightarrow{\gamma_I} D + R_I \\ R_I &\xrightarrow{\beta_I} I \\ R_I &\xrightarrow{\delta_{R_I}} \text{degradation} \\ I &\xrightarrow{\delta_I} \text{degradation} \end{aligned} \tag{1}$$

The immediate early gene product, I, binds the promoter of early and late genes to express the early and the late gene mRNAs, R_E, and R_L, with the expression rate γ_E and γ_L, respectively. The early gene mRNA is then translated to viral DNA polymerase, E, with translation rate β_E. The late gene mRNA is then translated to viral envelop, L, with the rate β_L. mRNAs of the early and late genes are degraded with degradation rate δ_{R_E} and δ_{R_L}, respectively. The viral polymerase and envelop is degraded with the rate δ_E and δ_L, respectively. The chemical reaction equation concerning the early and late gene expression is

$$\begin{aligned} D + I &\xrightarrow{\gamma_E} D + I + R_E \\ R_E &\xrightarrow{\beta_E} E \\ R_E &\xrightarrow{\delta_{R_E}} \text{degradation} \\ E &\xrightarrow{\delta_E} \text{degradation} \\ D + I &\xrightarrow{\gamma_L} D + I + R_L \\ R_L &\xrightarrow{\beta_L} L \\ R_L &\xrightarrow{\delta_{R_L}} \text{degradation} \\ L &\xrightarrow{\delta_L} \text{degradation.} \end{aligned} \tag{2}$$

The viral DNA, D, is replicated by the polymerase, E, with reaction rate constant α_1. The complete virion, V, is produced by the packaging of the viral DNA, D, by the envelope, L, with reaction rate constant α_2. Viral DNA and complete virion are degraded by the degradation rate δ_D and δ_V,

respectively. The reaction equation of the viral DNA replication and reproduction of the virion is

$$\begin{aligned} D + E &\xrightarrow{\alpha_1} 2D + E \\ D + L &\xrightarrow{\alpha_2} V \\ D &\xrightarrow{\delta_D} \text{degradation} \\ V &\xrightarrow{\delta_V} \text{degradation} \end{aligned} \tag{3}$$

The time change of the concentrations of viral DNA, D, immediate early, early and late gene mRNAs, R_I, R_E, R_L, immediate early, early and late gene products, I(transcription factor), E(viral DNA polymerase) and L(envelop), and the complete virion, V are then given by the low of mass action:

$$\begin{aligned} \frac{dD}{dt} &= \alpha_1 DE - \alpha_2 DL - \delta_D D \\ \frac{dR_I}{dt} &= \gamma_I D - \delta_{R_I} R_I \\ \frac{dI}{dt} &= \beta_I R_I - \delta_I I \\ \frac{dR_E}{dt} &= \gamma_E DI - \delta_{R_E} R_E \\ \frac{dE}{dt} &= \beta_E R_E - \delta_E E \\ \frac{dR_L}{dt} &= \gamma_L DI - \delta_{R_L} R_L \\ \frac{dL}{dt} &= \beta_L R_L - \alpha_2 DL - \delta_L L \\ \frac{dV}{dt} &= \alpha_2 DL - \delta_V V \end{aligned} \tag{4}$$

The abbreviations are summarized in Table 1. In this system, the initial concentration of viral DNA is D_0, and all other quantities are 0.

3 Function of the temporally ordered expression

The time course of the viral replication is obtained from the full dynamic system (4) as shown in Fig 2A. Our model can reproduce the temporal pattern of the viral gene expression. Therefore immediate early gene product is first accumulated. And the expression of early and late genes is

then activated by the immediate early gene product. It is reported that the peak of the synthesis of the immediate early gene product is from 2 to 4h after infection(Boehmer & Lehman, 1997). The expression of early gene is activated by the immediate early gene products. Protein synthesis of early gene reaches peak rates from 5 to 7h after infection. Viral DNA is replicated by the viral polymerase encoded on the early gene. Viral DNA synthesis begins shortly after the appearance of early gene product. Replicated viral DNA is encapsidated by the envelop protein encoded on the late gene to produce the complete virion. As a result, the concentration of virion finally increases. The parameters in our model is estimated by this reported temporal profile of HSV-1 replication. The summarized expression pattern of I, E and L is shown by bars in Fig 2A. Of course, the infected cell is disrupted by lytic process to release the virions before their concentration diverges to infinity.

We investigate how parameters affect the intracellular dynamics of HSV-1 replication. We found that the temporal order from early gene to late gene critically influences the dynamics of HSV-1 replication. γ_E and γ_L in our model represent the expression rate of the the early and the late genes. This ratio determines the temporally ordered expression of early and late gene. When γ_E is larger than γ_L, we can obtain the temporal profile from our model as well as experimental observation. As shown in Fig 2B, when γ_L is larger than γ_E, the late gene is expressed earlier than the early gene does. This expression pattern clearly contradicts to that observed in *in vitro* experiments. With this wrong order of expression, the concentration of virion converge to a certain fixed value. The concentration of DNA remain very small because the consumption of the viral DNA to produce the complete virion starts earlier than the positive feedback of viral DNA/polymerase production takes place. The viral DNA is necessary both as the template of the viral mRNAs and as the component of the complete virion. When the concentration of the viral DNA becomes 0, the reproduction of the virus is arrested. This result indicates that the temporally ordered expression of viral genes critically affects the growth rate of HSV-1. Sufficiently preceded expression of the early gene to the late gene enables HSV-1 to grow continuously in the host cell.

The concentration of virion after sufficiently long time has passed from the infection (30h after infection) is obtained when γ_E and α_1 are changed. Light color in Fig 3 indicates the region where the concentration of virion diverge to infinite within this time period. The threshold exist on the line where the product γ_E and α_1 become constant. To understand the mechanism how the dynamics of HSV-1 replication is drastically changed by the expression pattern of viral genes, we construct a simplified model of HSV-1 replication.

4 The simplification of the model

Now, we consider the initial phase of the infection to simplify the model. The initial concentration value of viral DNA is D_0 which remains until the replication of viral DNA has started. The early and the late gene mRNAs, R_E and R_L, are accumulated depending on the concentration of the immediate early gene product, I, from the initial concentration, $R_E = R_L = 0$. To consider $I - R_E$ and $I - R_L$ phase plane, R_E and R_L increases just below the null cline $R_E = \gamma_E DI/\delta_{R_E}$ and $R_L = \gamma_L DI/\delta_{R_L}$, respectively. On the other hand, R_I increase depends on the initial concentration of viral DNA, D_0, until D is increased by the replication. To consider $D - R_I$ phase plane, R_I increases towards the null cline $R_I = \gamma_I D/\delta_{R_I}$ from $(D_0, 0)$ as D hardly increases. After R_I reaches the null cline $R_I = \gamma_I D/\delta_{R_I}$, R_I increases just below this line. These yield

$$\begin{aligned} R_I &\simeq \frac{\gamma_I}{\delta_{R_I}} D \\ R_E &\simeq \frac{\gamma_E}{\delta_{R_E}} DI \\ R_L &\simeq \frac{\gamma_L}{\delta_{R_L}} DI. \end{aligned} \tag{5}$$

We also assume that the half lives of the viral proteins are much larger than the period of the initial phase we are considering, and therefore ignore their degradation. These simplifications reduce the

model (4) to

$$\begin{aligned}
\frac{dD}{dt} &= \alpha_1 DE - \alpha_2 DL \\
\frac{dI}{dt} &= \frac{\beta_I \gamma_I}{\delta_{R_I}} D \\
\frac{dE}{dt} &= \frac{\beta_E \gamma_E}{\delta_{R_E}} DI \\
\frac{dL}{dt} &= \frac{\beta_L \gamma_L}{\delta_{R_L}} DI - \alpha_2 DL \\
\frac{dV}{dt} &= \alpha_2 DL
\end{aligned} \tag{6}$$

The diagram of the intracellular replication represented by the simplified model is shown in Fig 4. This simplification is appropriate until viral DNA, mRNAs and proteins are sufficiently accumulated.

5 Analytical result

5.1 D, E and L as a function of I

Because the concentration of the viral DNA plays critical role determining the growth pattern of HSV-1, we examine the trajectory of D as a function of I to investigate the threshold value for the explosion of the virus. When D, E and L are differentiated with respect to I,

$$\begin{aligned}
\frac{\frac{dD}{dt}}{\frac{dI}{dt}} = \frac{dD}{dI} &= \frac{\alpha_1}{\gamma_I}\frac{\delta_{R_I}}{\beta_I} E - \frac{\alpha_2}{\gamma_I}\frac{\delta_{R_I}}{\beta_I} L \\
\frac{\frac{dE}{dt}}{\frac{dI}{dt}} = \frac{dE}{dI} &= \frac{\gamma_E}{\gamma_I}\frac{\delta_{R_I}}{\beta_I}\frac{\beta_E}{\delta_{R_E}} I \\
\frac{\frac{dL}{dt}}{\frac{dI}{dt}} = \frac{dL}{dI} &= \frac{\gamma_L}{\gamma_I}\frac{\delta_{R_I}}{\beta_I}\frac{\beta_L}{\delta_{R_L}} I - \frac{\alpha_2}{\gamma_I}\frac{\delta_{R_I}}{\beta_I} L.
\end{aligned} \tag{7}$$

By solving (7) with the initial concentration, $D = D_0$, $I = E = L = 0$ at $t = 0$, we obtain D, E and L as a function of I as follows:

$$\begin{aligned} D &= D_0 + \frac{\gamma_L\beta_L}{\alpha_2\delta_{R_L}}I - \frac{\gamma_L\beta_L}{2\delta_{R_L}}\frac{\delta_{R_I}}{\gamma_I\beta_I}I^2 + \frac{\alpha_1}{6}\left(\frac{\delta_{R_I}}{\gamma_I\beta_I}\right)^2\frac{\gamma_E\beta_E}{\delta_{R_E}}I^3 \\ &\quad + \left(-1 + \exp\left[-\frac{\alpha_2\delta_{R_I}}{\gamma_I\beta_I}I\right]\right)\frac{\gamma_I\beta_I}{\alpha_2^2\delta_{R_I}}\frac{\gamma_L\beta_L}{\delta_{R_L}} \\ E &= \frac{\delta_{R_I}}{2\gamma_I\beta_I}\frac{\gamma_E\beta_E}{\delta_{R_E}}I^2 \\ L &= \frac{\gamma_I\beta_I}{\alpha_2^2\delta_{R_I}}\frac{\gamma_L\beta_L}{\delta_{R_L}}\left(-1 + \exp\left[-\frac{\alpha_2\delta_{R_I}}{\gamma_I\beta_I}I\right]\right) + \frac{\gamma_L\beta_L}{\alpha_2\delta_{R_L}}I. \end{aligned} \tag{8}$$

Let $\xi_I = \gamma_I\beta_I/\delta_{R_I}$, $\xi_E = \gamma_E\beta_E/\delta_{R_E}$ and $\xi_L = \gamma_L\beta_L/\delta_{R_L}$. These are substituted into (8), we obtain D, E and L as follows:

$$\begin{aligned} D &= D_0 + \frac{\xi_L}{\alpha_2}I - \frac{\xi_L}{2\xi_I}I^2 + \frac{\alpha_1\xi_E}{6\xi_I^2}I^3 + \frac{\xi_I\xi_L}{\alpha_2^2}\left(-1 + \exp\left[-\frac{\alpha_2}{\xi_I}I\right]\right) \\ E &= \frac{\xi_E}{2\xi_I}I^2 \\ L &= \frac{\xi_L}{\alpha_2^2\xi_I}\left(-1 + \exp\left[-\frac{\alpha_2}{\xi_I}I\right]\right). \end{aligned} \tag{9}$$

The trajectory of D in (D,I) phase plane is shown in Fig 5. When D becomes always positive plotted by solid line in Fig 5, the immediate early gene continues to be expressed and I continues to increase. As a result, V diverges to infinity (see section5.3.1). Once D becomes 0 plotted by dotted line in Fig 5, the reproduction of virus is arrested because all viral gene expressions and production of the virion stop. We investigate the condition under which D becomes always positive and leads to the explosion of the virus.

5.2 The condition for the explosion of the virus

5.2.1 Small I

Now we consider the initial phase of the infection. We expand D of (9) in Taylor series with respect to I because the concentration of the immediate early gene products, I, is still small. Approximated

D for small I is

$$\begin{aligned} D_0 &= D_0 + \frac{\alpha_1\xi_E - \alpha_2\xi_L}{6\xi_I^2} I^3 \\ &= D_0 + \frac{1}{6}\left(\frac{\delta_{R_I}}{\gamma_I\beta_I}\right)^2 \left(\alpha_1\frac{\gamma_E\beta_E}{\delta_{R_E}} - \alpha_2\frac{\gamma_L\beta_L}{\delta_{R_L}}\right) I^3. \end{aligned} \tag{10}$$

As shown in Fig 6, approximate D defined in (10) well agrees with the exact D defined in (8). From (10), we obtain the condition for the explosion of the virus: $\alpha_1\gamma_E\beta_E/\delta_{R_E} > \alpha_2\gamma_L\beta_L/\delta_{R_L}$. We confirm whether this condition obtained from the analytical result of simplified model agree with the threshold obtained from the full dynamic system (4). As shown in Fig 3, the threshold obtained from full dynamic model exist on the line $\alpha_1\gamma_E = \alpha_2\gamma_L\delta_{R_E}\beta_L/\beta_E\delta_{R_L} =$ constant. This result indicates that the simplified model is appropriate for estimating the replication pattern of HSV-1, explosive growth or growth arrest. The growth pattern of HSV-1, explosive or arrest, is determined by whether positive feedback from viral DNA to early gene product do work or not. The preceded accumulation of early gene product contributing to replicate the viral DNA to late gene product contributing to consume the viral DNA to produce the complete virion critically affects the growth pattern of HSV-1.

In this condition, γ_E and γ_L represent the expression rate of early and the late gene. On the other hand, β_E/δ_{R_E} and β_L/δ_{R_L} represent the translation rate of the early and the late gene mRNAs until degradation. The expression activation of early gene for large γ_E, efficient translation of early gene product for large β_E and the stabilization of the early gene mRNA for small δ_{R_E} enhance HSV-1 replication through the predominant accumulation of early gene product.

Now we focus on the expression ratio of the early to the late gene. When the translation and degradation rate of viral mRNAs are the same, $\gamma_E/\gamma_L > \alpha_2/\alpha_1$ is the condition for the viral explosion. When γ_E/γ_L is larger than α_2/α_1, D is always positive and monotonically increase with time. Otherwise, D decreases and becomes 0 when sufficiently long time has passed. γ_E and γ_L represent the activity of the early and the late gene promoters, respectively. This result indicates that the growth pattern of HSV-1, the explosive growth or the growth arrest, is determined by the

activity ratio of the early gene promoter to that of the late gene promoter. α_1 and α_2 are the reaction rates of the viral DNA replication and the production of the complete virion by the encapsidation, respectively. The activity ratio of the early gene promoter to the late gene promoter must be larger than the ratio of the reaction rate of the encapsidation to that of the viral DNA replication for the explosive growth of HSV-1. The structural difference of the early and the late gene promoters, high activity with many binding sites to transcription factor in the early gene promoter in contrast to a low activity with a few binding sites in the late gene promoter, corresponds to large γ_E and small γ_L in our model. This structural difference between the early and the late gene promoters of HSV-1 are suitable for promoting viral growth.

5.2.2 Large I

Up to now, we consider wheather or not the reproduction of HSV-1 is arrested within sufficiently short period. Next we examine the case when I becomes sufficiently large. When I becomes sufficiently large, the exponential term of D in (8) becomes sufficiently small and can be ignored. We approximate D for large I as follows:

$$D = D_0 - \frac{\xi_I \xi_L}{\alpha_2^2} + \frac{\xi_L}{\alpha_2} I - \frac{\xi_L}{2\xi_I} I^2 + \frac{\alpha_1 \xi_E}{6\xi_I^2} I^3 \tag{11}$$

As shown in Fig 6, the result obtained from (10) and (11) agree well with the exact solution of D for large I. Eq. (11) is always positive if I is sufficiently large. This indicates that once I becomes sufficiently large under the condition for the explosion of the virus at initial phase of infection, D becomes positive anyway. Therefore $\alpha_1 \gamma_E \beta_E / \delta_{R_E} > \alpha_2 \gamma_L \beta_L / \delta_{R_L}$ gives the sufficient condition for the explosion of the virus. As shown in Fig 6, I becomes sufficient large for (11) that correspond well to exact solution of D after (10) is equal to (11). Let (10) be equal to $\alpha_1 \xi_E I^3 / 6\xi_I^2$, we obtain I^* to switch from (10) to (11).

$$\begin{aligned} D_0 + \frac{\alpha_1 \xi_E - \alpha_2 \xi_L}{6\xi_I^2} I^{*3} &= \frac{\alpha_1 \xi_E}{6\xi_I^2} I^{*3} \\ I^* &= \left(\frac{6\xi_I^2}{\alpha_2 \xi_L} D_0 \right)^{\frac{1}{3}} \end{aligned} \tag{12}$$

6 The replication speed of HSV-1

6.1 The waiting time for the virus explosion

In this section, we investigate the replication speed of HSV-1 when γ_E is larger than the threshold level for the explosion of the virus. We obtain the waiting time for the virus explosion from time dependent solution of D. Substituting approximated D for large I, $D = \alpha_1 \xi_E I^3 / 6\xi_I^2$, into D of (6), the time dependent solution of I is obtained.

$$\begin{aligned} \frac{dI}{dt} &= \xi_I D \\ &= \frac{\alpha_1 \xi_E}{6\xi_I} I^3 \\ I &= \sqrt{\frac{3\xi_I}{\alpha_1 \xi_E (t_c - t)}} \end{aligned}$$

Now t_c is waiting time for virus explosion.

$$t_c = \frac{3\xi_I}{I_0^2 \alpha_1 \xi_E} \tag{13}$$

The waiting time for viral explosion is obtained substituting I^* into (13).

$$\begin{aligned} t_c &= \left(\frac{3\alpha_2^2 \xi_L^2}{4 D_0^2 \xi_I \xi_E^3} \right)^{\frac{1}{3}} \\ &= \left(\frac{3\alpha_2^2}{4\alpha_1^3 D_0^2} \frac{\delta_{R_I}}{\gamma_I \beta_I} \left(\frac{\delta_{R_E}}{\gamma_E \beta_E} \right)^3 \left(\frac{\gamma_L \beta_L}{\delta_{R_L}} \right)^2 \right)^{\frac{1}{3}} \end{aligned} \tag{14}$$

This result indicates that the waiting time for viral explosion becomes short when initial infection dose of HSV-1 designated by D_0 becomes large. The waiting time obtained from the simplified model is underestimated as compared with the result from full dynamic system (4). The simplification becomes inappropriate as the concentration of viral DNA, mRNAs, and proteins are sufficiently large so that the degradation term cannot be ignored. But the result that the waiting time becomes shorter as D_0 becomes large is qualitatively conserved in the full dynamic system (4).

6.2 effect of the ratio γ_E/γ_L for the replication speed

Next, we investigate the effect of the ratio γ_E/γ_L for the replication speed of HSV-1. The time course of the concentration of virion is numerically calculated from full dynamics system (4) when γ_L is fixed and γ_E is increased. V increases more rapidly as γ_E increases as shown in Fig 7. This result indicates that the replication speed of the HSV-1 is accelerated as the activity of the early gene promoter reflecting the production rate of early gene mRNA becomes large.

6.2.1 The mutation affecting the transcriptional activities of early and late gene promoter

Here, we consider the point mutation that increases or decreases the promoter activity of the early and late gene of HSV-1. When a new binding site to which the transcription factor binds is produced in the promoter by the mutation, the activity of the promoter is increased. Conversely, the promoter activity is decreased by a random mutation at the binding site. Four kinds of mutations are considered. In the mutant designated by E^+, a new binding site is produced by the mutation in the early gene promoter. γ_E of E^+ becomes larger ($0.55[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$ in Fig 8) than that of the wild type ($0.5[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$ in Fig 8). In the mutant designated by E^-, the binding site of early gene promoter is disrupted by the mutation. γ_E of E^- becomes smaller ($0.45[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$) than that of the wild type. Similar mutation is considered about the late gene promoter. In the mutant designated by L^+ and L^-, the binding affinity of late gene promoter is increased or decreased, respectively. γ_L of L^+ (L^-) is larger ($0.15[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$) (smaller ($0.05[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$)) than that of the wild type. In these four mutants and wild type, E^+ grows most rapidly as shown in Fig 8. Interestingly, the decreased binding affinity at late gene promoter (L^-) *accelerate* the viral growth, and the increased affinity at late gene promoter (L^+) *decelerate* it. The mutants, E^- and L^+ grow slower than the wild type. This result indicates that the reproduction speed becomes faster by the mutation that creates the new binding site in the early gene promoter.

7 Effect of the limitation of the intracellular resources

In our model analyzed so far, there are no limitation of the virus growth. In actual cell, it is impossible for the concentration of virion to diverge to infinity. The intracellular production rate is bounded by resources for virus replication such as nucleic acids and amino acids. It is important to know whether the condition for growth arrest/explosive growth is affected by the constraint of the intracellular resources. To investigate the effect of these limitation of the intracellular resources, we expand our model to take the dynamics of the resources into consideration. The model including the time change of the concentration of amino acid, deoxyribonucleic acid and ribonucleic acid are designated A, N_d and N_r are:

$$\begin{aligned}
\frac{dD}{dt} &= \alpha_1' DEN_d - \alpha_2 DL - \delta_D D \\
\frac{dR_I}{dt} &= \gamma_I' DN_r - \delta_{R_I}' R_I A \\
\frac{dI}{dt} &= \beta_I' R_I A - \delta_I I \\
\frac{R_E}{dt} &= \gamma_E' DIN_r - \gamma_{R_E}' R_E A \\
\frac{dE}{dt} &= \beta_E' R_E A - \delta_E E \\
\frac{dR_L}{dt} &= \gamma_L' DIN_r - \gamma_{R_L}' R_L A \\
\frac{dL}{dt} &= \beta_L' R_L A - \alpha_2 DL - \delta_L L \\
\frac{dV}{dt} &= \alpha_2 DL - \delta_V V \\
\frac{dA}{dt} &= \lambda_1 - D(\beta_I' R_I + \beta_E' R_E + \beta_L' R_L) A \\
\frac{dN_d}{dt} &= \lambda_2 - \alpha_1' DEN_d \\
\frac{dN_r}{dt} &= \lambda_3 - \gamma_I' DN_r - \gamma_E' DIN_r - \gamma_L' DIN_r. \qquad (15)
\end{aligned}$$

Here, λ_1, λ_2 and λ_3 are the constant supply of amino acid, deoxyribonucleic acid and ribonucleic acid. The parameters use in the previous sections are re-defined to adapt to the change of the interaction among three molecules as $\alpha_1 = \alpha_1' N_d(0), \gamma_I = \gamma_I' N_r(0), \beta_I = \beta_I' A(0)$.

The concentration of virion after sufficiently long time has passed (100h after infection)

with various ratio γ'_E/γ'_L are shown in Fig 9. The final concentration of virion hardly increase when the ratio γ'_E/γ'_L is small. The concentration of deoxyribonucleic acid does not change from its initial concentration in this case. This result correspond to the growth arrest caused by the consumption of genomic DNA of HSV-1 by the excessive expression of the late gene product. The final concentration of virion suddenly increases when the ratio γ'_E/γ'_L becomes more than 0.4. Though this threshold becomes slightly smaller as compare to the ratio $\alpha_2/\alpha'_1 N_d(0) = 0.5$, this threshold corresponds to that in the absence of limitation, $\gamma_E/\gamma_L > \alpha_2/\alpha_1$.

If the ratio γ'_E/γ'_L is further increased past the threshold $\alpha_2/\alpha'_1 N_d(0)$, the final concentration of virion attains the maximum, and then decreases towards $\gamma'_E/\gamma'_L \to \infty$. All the amino acids are converted to viral proteins, immediate early, early and late gene products at equilibrium. As the ratio γ'_E/γ'_L becomes larger, the final concentration of virion is decreased by the shortage of envelop. Thus there is an optimal ratio γ'_E/γ'_L, which is never expected in the model without limitation, where the growth speed of virus monotonically increase with γ'_E/γ'_L. With a very large γ'_E/γ'_L ratio, the virion accumulates quickly but hits a lower saturation level than when γ'_E/γ'_L is intermediate (Fig 10).

8 Discussion

Herpesviridae is a major family of the DNA viruses causing many human diseases. *Herpesviridae* is important for the public health. Among *Herpesviridae*, HSV-1 is most extensively studied about its replication and the gene expression as a typical system of DNA viruses. In the past studies about the viral gene expression, the mechanism regulating the temporally ordered expression of the viral gene is focused. However, we focus on a functional role of this temporal pattern of the viral gene expression. We construct a simple mathematical model for the intracellular replication of HSV-1.

Our model is based on the biological information about the process of viral replication and

gene expression of HSV-1. The intracellular replication of HSV-1 is described as a chemical reaction equations. In our model, the concentrations of mRNA and protein of the classified genes into each group, immediate early, early and late genes, are addressed as one variable, because the time course of these gene products within group are common to show the characteristic temporal expression pattern. When the expression rate, translation rate of protein and half life of mRNA and protein within group are almost same, the variance of the concentrations of mRNAs and proteins within group is small to observe the temporally ordered expression pattern that enable the classification of viral genes. It is investigated that how the order of the viral gene expression affects the intracellular dynamics of the viral components such as the viral DNA, mRNAs and the proteins.

To compare Fig 2A with Fig 2B, the replication pattern of HSV-1 is drastically changed when the temporal pattern of the viral gene expression is changed. When the early genes expression proceeds as normally observed in the infection of HSV-1, the concentration of virion diverges to infinity within a finite time period. This is the eexplosivef growth of the virus. Though it cannot happen in the actual infection of HSV-1, the late gene expression proceeds, the concentration of the virus converges to a certain positive value. This is the growth arrest. Whether eexplosionf or earrestf are decided by the order of the viral gene expression. Almost all early genes encode the component of the viral DNA polymerase and DNA binding protein regulating the DNA replication of the virus. Sufficient proceeded expression of the viral DNA polymerase enable HSV-1 to grow continuously in the host.

To analytically investigate the dynamics of viral replication, our model is simplified by considering the initial phase of the infection. The condition for the 'explosion' is obtained from simplified model. From (8), sufficient condition for 'explosion' is $\alpha_1\gamma_E\beta_E/\delta_E$ is larger than $\alpha_2\gamma_L\beta_L/\delta_L$. γ_E and γ_L represent the promoter activity of early and late gene. β_E/δ_E and β_L/δ_L represent the translation rate until degradation of mRNAs of early gene and late gene. This condition implies that predominant accumulation of early gene product is suitable for the explosive replication of HSV-1. This condition for the explosive growth of HSV-1 analytically obtained from simplified

model well agree with the threshold obtained from the full dynamic model (4). The viral DNA replication in early phase of the infection critically affects the dynamics of HSV-1 replication. The concentration of the complete virion is explosively increased by the effect of the positive feedback from DNA replication to early gene expression.

To focus on the expression ratio of the early to late gene, the condition is simplified as γ_E/γ_L is larger than α_2/α_1 when the translation rate and degradation rate of viral mRNAs are the same. Large expression ratio of early gene as compared with late gene is suitable for the explosive replication of HSV-1. The expression ratio of early to late genes critically depends on the structure of early and late gene promoters. It is well understood that there are many binding motifs, such as TAATGARAT, to which the transcription factors bind on the early gene promoter in contrast to a few binding motifs on the late gene promoter. Structural difference in the early and the late gene promoters maintain the continuous growth of virus by preceding expression of early gene to late gene. This introduces the temporally ordered expression pattern as we experimentally observe. Large γ_E and small γ_L correspond to the prevention of the growth arrest.

To analytically understand the dynamics of viral replication, it is important for considering the therapy of viral infection. From the result obtained from our model, the target of the therapeutics against HSV-1 infection is predicted. It can be expected that the concentration of the complete virion is drastically decreased by the inhibition of preceded accumulation of early gene product. DNA replication by early gene product is inhibited to reduce α_1. Expression of early gene is inhibited to reduce γ_E. Translation of early gene mRNA is inhibited to reduce β_E. And early gene mRNA is destabilized to increase δ_{R_E}. The positive feedback cannot work when the condition for the explosive replication of HSV-1, $\alpha_1\gamma_E\beta_E/\delta_{R_E} > \alpha_2\gamma_L\beta_L/\delta_{R_L}$, is not satisfied.

For example, it is reported that siRNAs targeting UL39 gene of HSV-1 can prevent the replication of HSV-1(Zhe *et al.*, 2008). UL39 gene is classified as early gene and encodes the large subunit of ribonucleotide reductase designated by ICP6. UL39 mRNA is degraded by RNAi in this

experiment. This corresponds that δ_{R_E} becomes large in our model.

Next, we investigate how the promoter activity of early and late genes influences the replication speed of the virus when γ_E becomes larger than the threshold level for the explosion of the virus. We obtain the time course of the concentration of the virion with various γ_E from the full dynamic system (4). As shown in Fig 7, the replication speed of the virus becomes faster as the activity of the early gene promoter becomes higher. Large γ_E not only prevents the growth arrest but also accelerates the reproduction speed of HSV-1.

Here, we consider the mutation in the early or the late gene promoter that changes the binding affinity of the promoter to the transcription factor. Four kinds of mutations are considered that increase or decrease of the binding affinity of the early or the late gene promoters, designated by E^+, E^-, L^+ and L^-. The mutant E^+, in which the early gene promoter has a new binding site with larger γ_E grows more rapidly than wild type. Interestingly, mutant L^- in which the late promoter lose the binding affinity with smaller γ_L grows more rapidly than wild type. These results indicate that the structural difference in early gene and late gene promoters might be selectively maintained through the replication speed of the virus. The viral genes of HSV-1 are clearly subdivided by the temporal order of the expression. The temporal profile of viral gene expression is different among DNA viruses(Gonçalves & de Vries, 2006). In HSV-1 replication, it is indicated that rate-limiting step of virion production is encapsidation of viral DNA (Koyama & Uchida, 1988). The viral DNA synthesis measured by incorporation of [^{3}H]thymidine into HSV-1 DNA began 3 h after infection. Newly synthesized DNA is encapsidated by capsid protein encoded on the late gene to produce the nucleocapsid 2 h later than the viral DNA synthesis. The appearance of infectious progeny virus coincide with that of nucleocapsid. This precedence of DNA synthesis to encapsidation by late gene product might correspond to both continuous and fast replication of virus in the host.

Finally the constraint of cellular resources such as amino acids and nucleic acids is considered. It is impossible for virus to infinitely grow in actual cell, because the intracellular resources

are limited. Therefore, the effect of the limitation of these resources is investigated. Our model is expanded to include the dynamics of amino acids, deoxyribonucleic acids and ribonucleic acids. The replication of virus stops when all these resources are consumed. The concentration of virion suddenly increase when the ratio γ'_E/γ'_L nearly exceeds the threshold ratio for explosion, $\alpha_2/\alpha'_1 N_d(0)$. α_1. This threshold ratio $\gamma_E/\gamma_L > \alpha_2/\alpha_1$ is appropriate under the limitation of the resources.

Differently from the no limitation, the final concentration of the virion decreases when γ'_E/γ'_L further increases past the threshold. The amino acids are converted to both the early and late gene products. The production of the envelop is decreased by the excessive production of the viral DNA polymerase. As a result, there is an optimal ratio γ'_E/γ'_L. This optimal ratio is determined by the relationship between the speed and efficiency of the virus replication. With a large γ'_E/γ'_L ratio, the virion accumulate quickly but final saturation level becomes low.

In the host cell, duration of the viral replication is determined by the timing for the death of infected cell. It is reported that the apoptosis is positively or negatively regulated when cell is infected by the various viruses(Everett & McFadden, 1999; Benedict *et al.*, 2002). For example, gamma herpesviruses and herpes simplex viruses induce or inhibit apoptosis through the BCL-2 homologs which is key mediator of apoptotic signal transduction(Hardwick & Bellows, 2003; Sciortino *et al.*, 2006). The waiting time for apoptosis plays critical role determining the optimal ratio of γ'_E/γ'_L. The replication speed is more important for the virus inducing apoptosis than the efficiency of the replication because the waiting time for the apoptosis becomes short. And then, the ratio γ'_E/γ'_L increases. Inversely, the ratio γ'_E/γ'_L of the virus inhibiting the apoptosis becomes close to the threshold $\alpha_2/\alpha'_1 N_d(0)$ to increase the replication efficiency. The activity of early and late gene promoter are dependent on the number of SP-1 binding site tandemly repeated in the promoter. The number of SP-1 site in the early gene promoter is larger than that in late gene promoter, but it is much smaller than that in the immediate early gene promoter(Rajcáni *et al.*, 2004). The number of SP-1 site in the early gene promoter is restricted to increase the efficiency of the replication of the virus.

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Appendix

The sufficient conditon for explosion is generally indicated. From (9),

$$\begin{aligned}
\frac{\partial D}{\partial I} &= \frac{\xi_L}{\alpha_2} - \frac{\xi_L}{\xi_I} I + \frac{\alpha_1 \xi_E}{2\xi_I^2} I^2 - \frac{\xi_L}{\alpha_2} \exp\left[-\frac{\alpha_2}{\xi_I} I\right] \\
\frac{\partial^2 D}{\partial I^2} &= -\frac{\xi_L}{\xi_I} + \frac{\alpha_1 \xi_E}{\xi_I^2} I + \frac{\xi_L}{\xi_I} \exp\left[-\frac{\alpha_2}{\xi_I} I\right] \\
\frac{\partial^3 D}{\partial I^3} &= \frac{\alpha_1 \xi_E}{\xi_I^2} + \frac{\alpha_2 \xi_L}{\xi_I^2} \exp\left[-\frac{\alpha_2}{\xi_I} I\right] \\
&= \frac{1}{\xi_I^2}\left(\alpha_1 \xi_E - \alpha_2 \xi_L \exp\left[-\frac{\alpha_2}{\xi_I} I\right]\right) \qquad \text{(A1)}
\end{aligned}$$

If $\alpha_1 \xi_E = \alpha_1 \gamma_E \beta_E / \delta_{R_E}$ is larger than $\alpha_2 \xi_L = \alpha_2 \gamma_L \beta_L / \delta_{R_L}$, $\partial^3 D / \partial I^3$ is always positive for all $I \geqslant 0$ from (A1). Therefore, $\partial^2 D / \partial I^2$ is monotonically increase for all $I \geqslant 0$. Because $\partial^2 D / \partial I^2 = 0$ when $I = 0$, $\partial^2 D / \partial I^2 \geqslant 0$ for all $I \geqslant 0$. D is convex and monotonically increases to infinity with $\alpha_1 \gamma_E \beta_E / \delta_{R_E} > \alpha_2 \gamma_L \beta_L / \delta_{R_L}$.

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	immediate early	early	late
mRNA	R_I	R_E	R_L
protein	I	E	L
transcription	γ_I	γ_E	γ_L
mRNA degradation	δ_{R_I}	δ_{R_E}	δ_{R_L}
translation	β_I	β_E	β_L
protein degradation	δ_I	δ_E	δ_L

Table 1: abbreviations

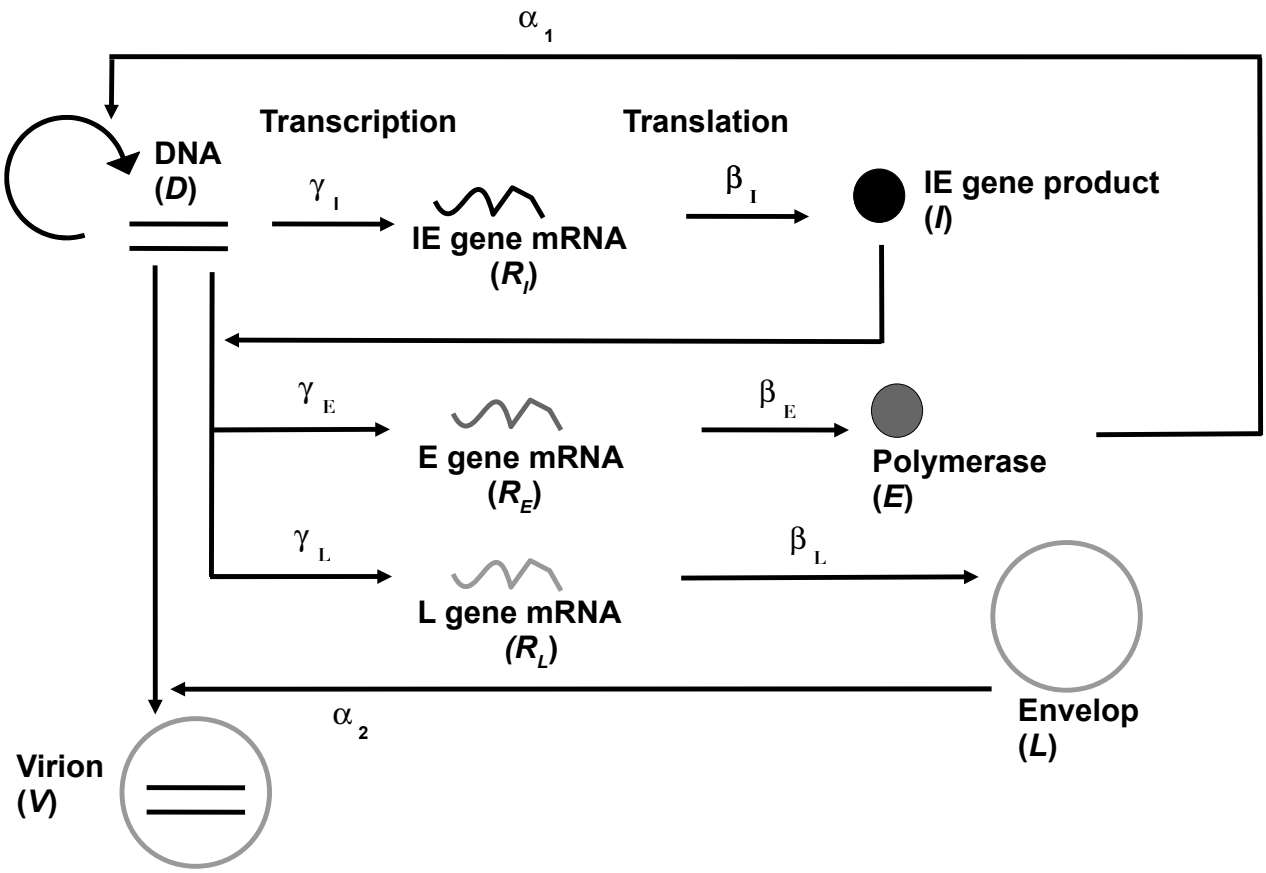

Figure 1: The intracellular reproduction of HSV-1. The intracellular reproduction of HSV-1 is schematically shown. The reproduction of HSV-1 has started when the viral DNA invades into the host cell. The immediate early gene is expressed without a new protein synthesis. The immediate early gene product activates the expression of the early and late genes. Early gene and late gene encode the viral DNA polymerase and the envelope of the viral particle, respectively. The viral DNA is replicated by the polymerase. The complete virion is produced by the interaction between the viral DNA and the envelope.

A

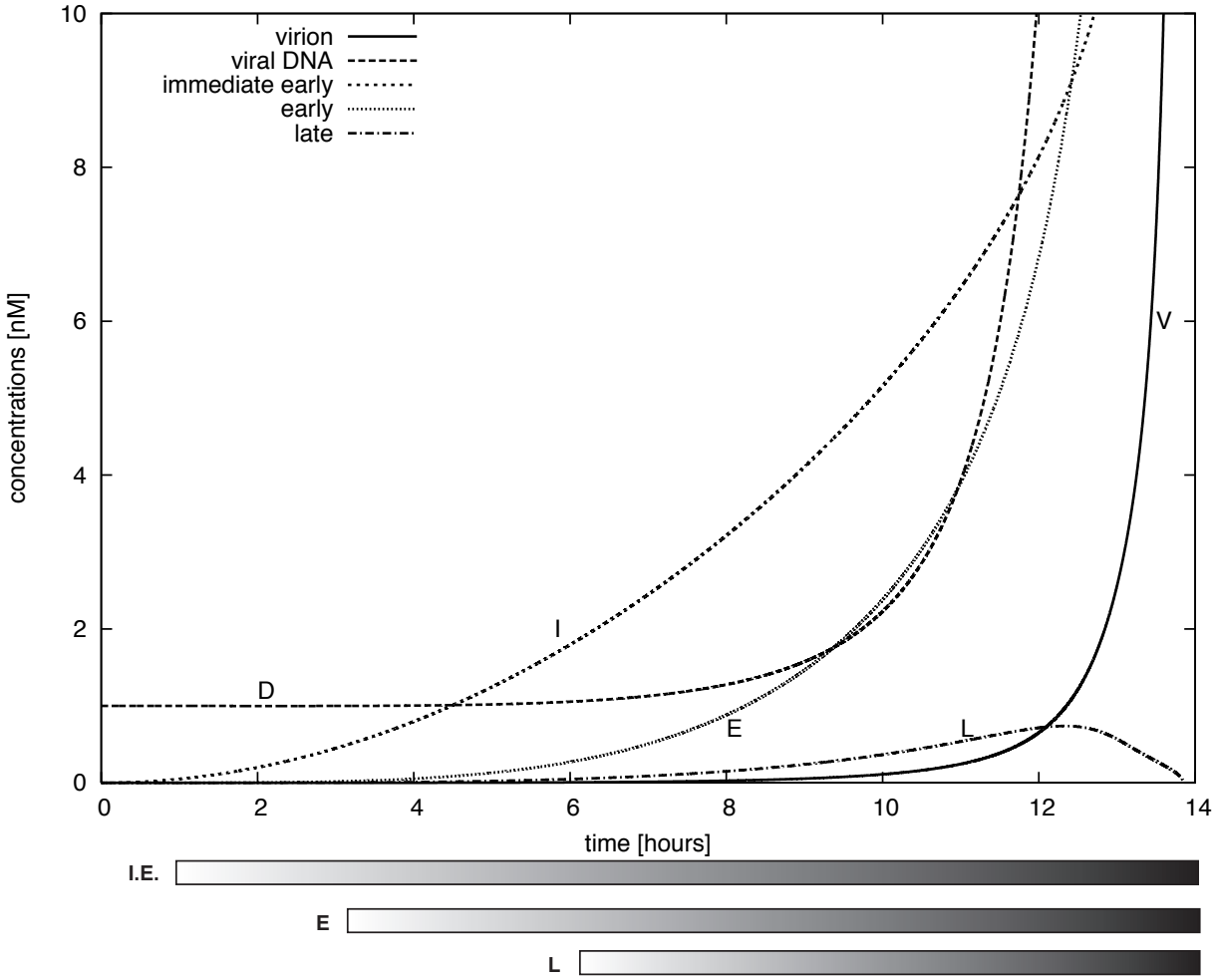

Figure 2: The time course of the concentrations of viral products, the viral DNA, the viral proteins and the virion from full dynamic system (4). To avoid the complication, the concentrations of mRNAs are excluded in this graph. The summarized expression pattern of the viral gene is shown by the bars under the graph. The viral DNA invades the host cell at time 0 with an initial concentration $D_0 = 1.0$. The immediate early gene is expressed and I increases. The immediate early gene product activates the expression of both the early and the late gene. In this graph, γ_1 is larger than γ_2. The early gene is predominantly expressed. E increases faster than L. The viral DNA is replicated by the polymerase, and then D incresaes. The virion is produced by the interaction between the viral DNA and the envelope, and then V increases. V diverges to infinite within a finite period. Parameters : $\gamma_I = 1.0[\mathrm{h}^{-1}]$, $\alpha_1 = 0.2[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\alpha_2 = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\gamma_E = 0.5[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\gamma_L = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\beta_I = \beta_E = \beta_L = 0.1[\mathrm{h}^{-1}]$ and $\delta_D = \delta_I = \delta_E = \delta_L = \delta_V = \delta_{R_I} = \delta_{R_E} = \delta_{R_L} = 0.001[[\mathrm{h}^{-1}]$. Parameters are estimated by the reported temporal profile of HSV-1 replication(Boehmer & Lehman, 1997).

B

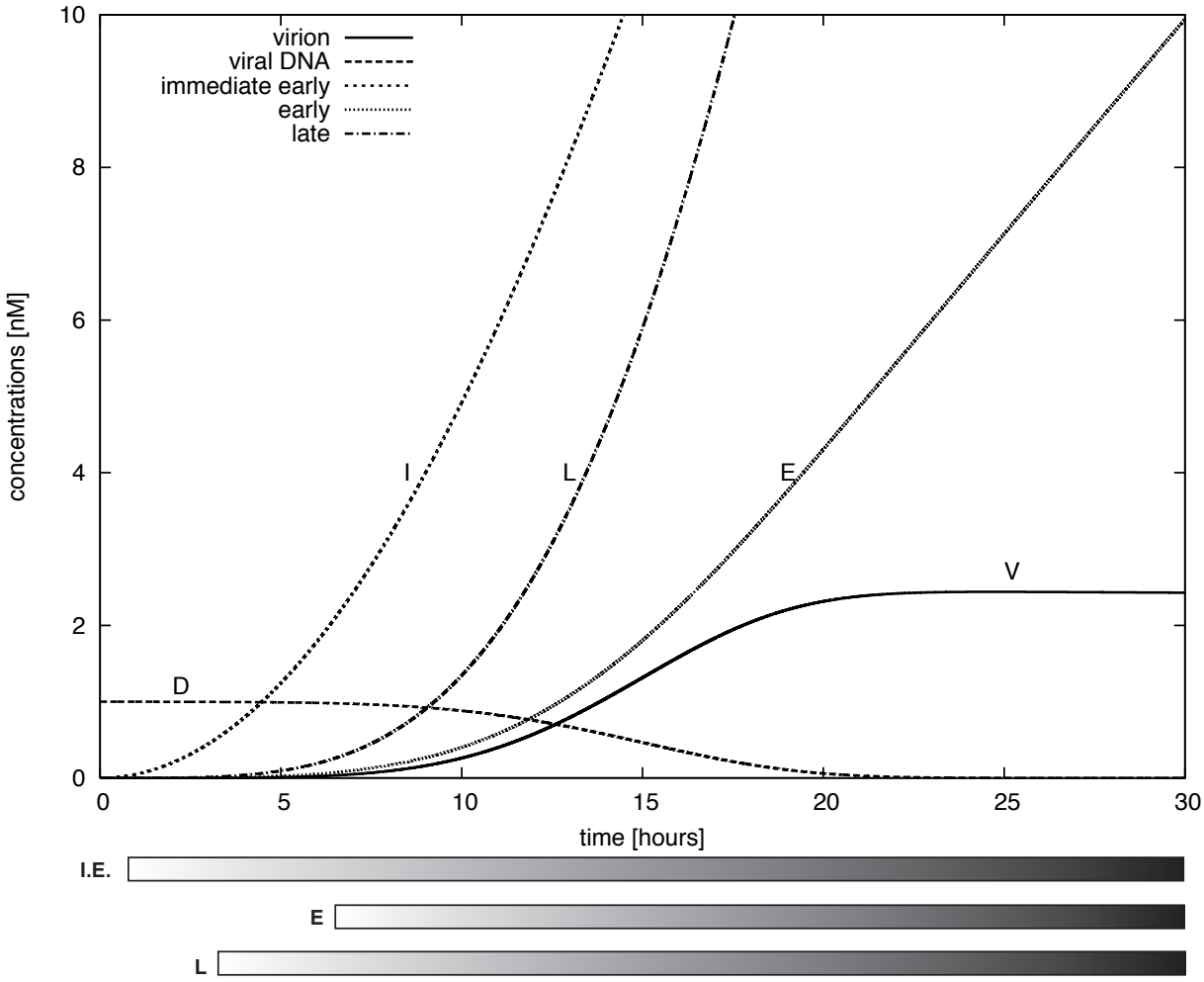

Figure 2: The time course of the concentrations of viral products, when ratio γ_E/γ_L is changed. The timing of the viral gene expression is changed. The late gene is predominatly expressed as compare to the early gene. The concentration of the viral DNA is decreased because the consumption of the viral DNA to produce the virion is larger than the replication. When D becomes 0, the reproduction of the virus is arrested. As a result, the concentration of the virion converge to a ceratin positive value. This result indicate that the timing of the temporally ordered expression of viral genes critically affects the growth pattern of the virus. Parameters are the same in Fig 2, except γ_E and γ_L. $\gamma_E = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\gamma_L = 0.4[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\gamma_E/\gamma_L = 0.25$.

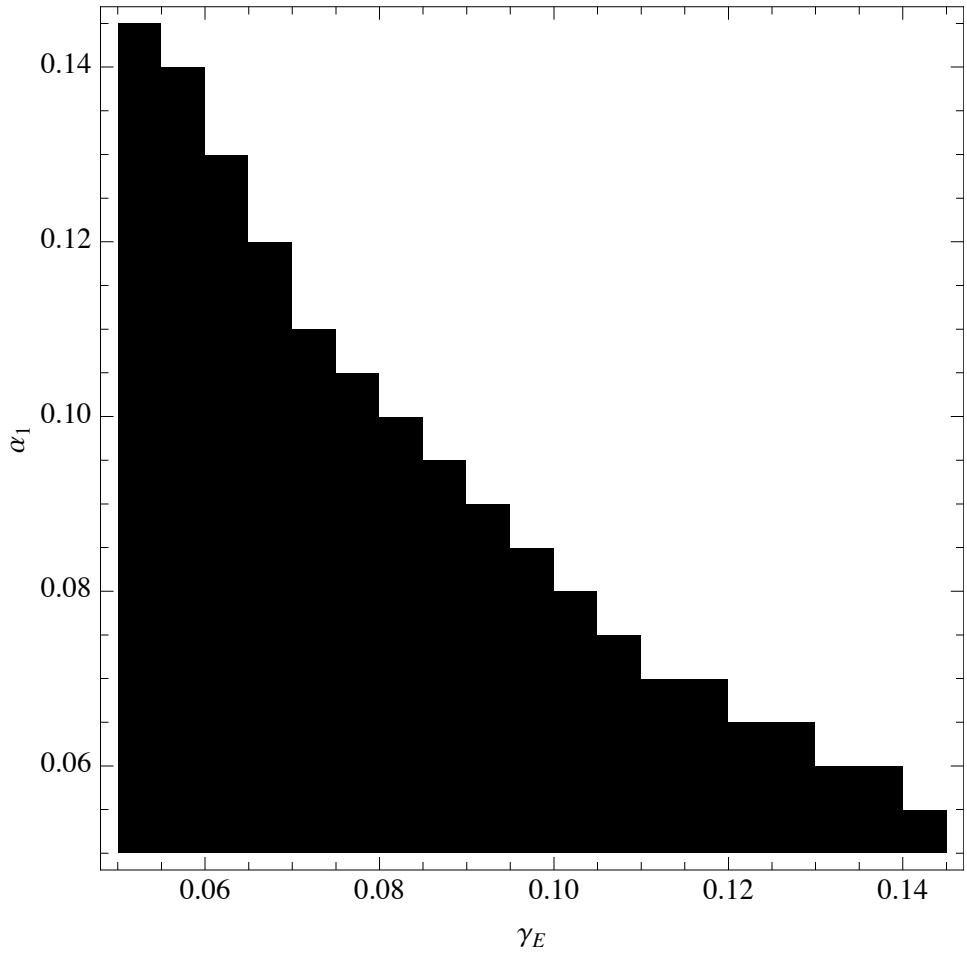

Figure 3: The density plot of the concentration of virion at a certain fixed time. Bright color in this graph shows the region where the concentration of virion diverges to infinite within a finite time scale. The horizontal and the vertical axis indicate γ_E and α_1, respectively. The threshold for explosion of virus is well agree with the analytical result, $\alpha_1 = \alpha 2\gamma_L/\gamma_E$. Parameters are $\alpha_2 = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, $\gamma_L = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$. α_2 and γ_L are fixed. γ_E and α_1 are changed from 0.05 to 0.145 $[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$.

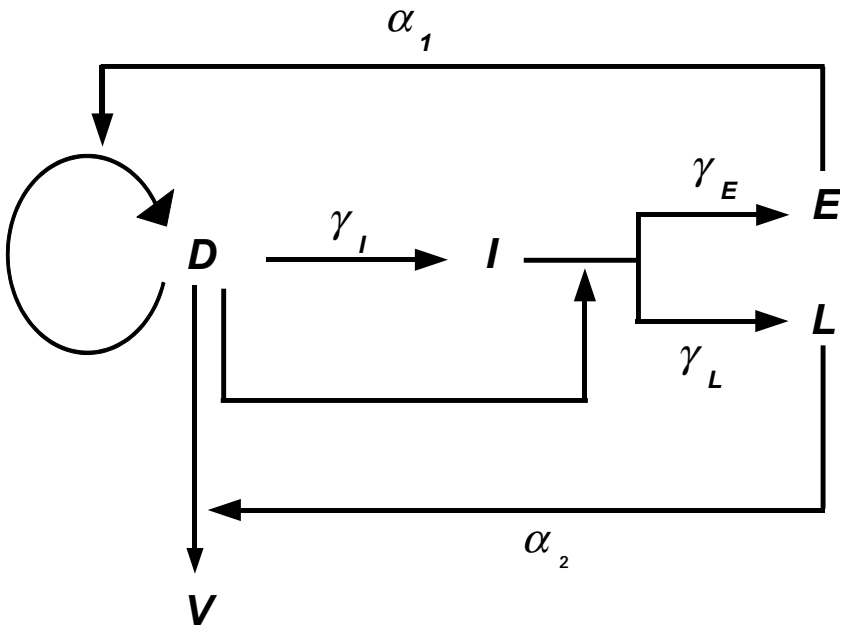

Figure 4: The diagram of the reproduction of the HSV-1 in simplified model. To consider the initial phase of the infection, we can ignore the time change of the viral mRNA concentrations. The gene product coded by the immediate early gene is expressed with production rate μ_1. It activates the expression of the early and the late gene expression with production rate γ_1 and γ_2, respectively. The early gene product replicates the viral DNA with reaction rate α_1. Complete virion is produced by the interaction between the viral DNA and the envelope with reaction rate α_2.

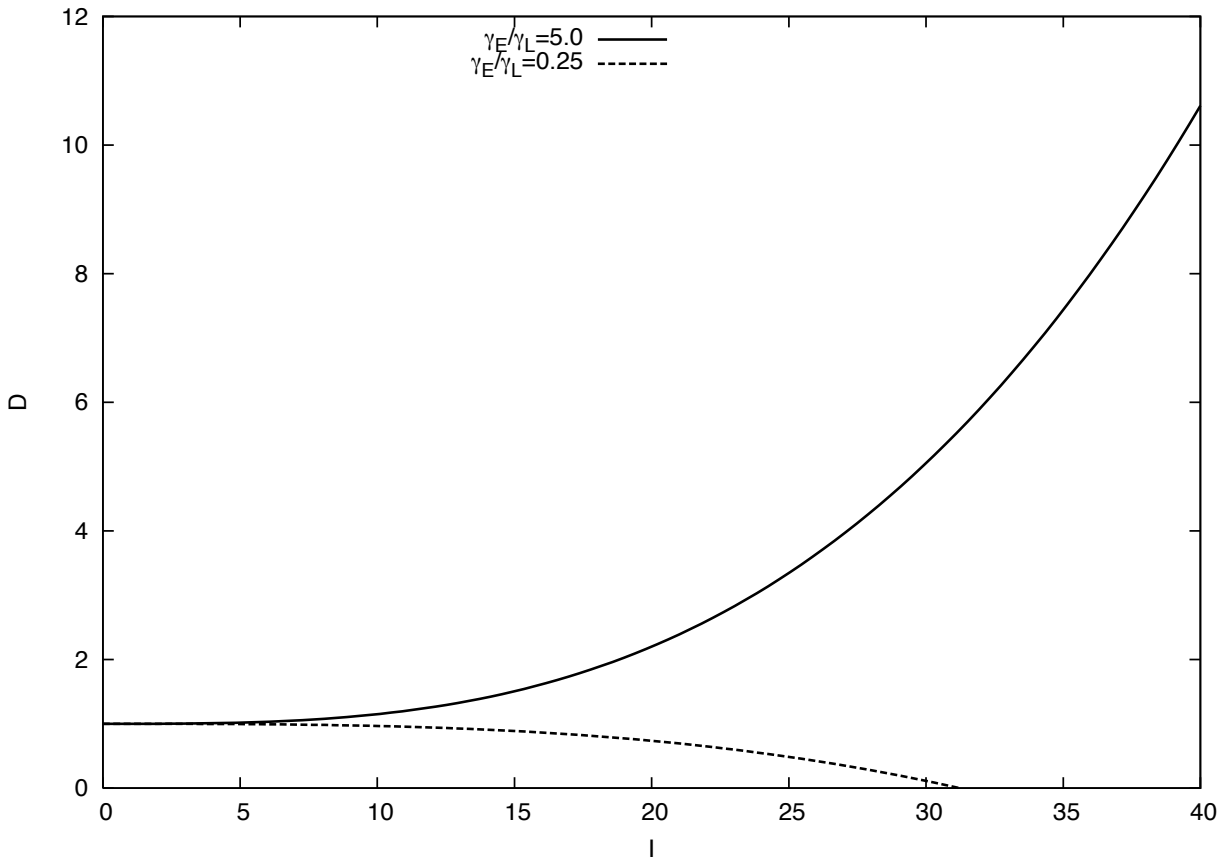

Figure 5: The trajectory of D as a function of I. The trajectory of D as a function of I with various $\gamma_E 1$ and γ_L are plotted. When D is positive as shown by the solid line, the immediate early gene is expressed and I continues to increase. Once D becomes 0 as shown by the dotted line, all viral gene expressions stop and the reproduction of HSV-1 is arrested. The growth pattern of HSV-1, the explosion or arrest, is determined by the ratio γ_E/γ_L. Parameters are the same in Fig 2 when $\gamma_E/\gamma_L = 5.0$. $\gamma_E = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$ and $\gamma_L = 0.4[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$, when $\gamma_E/\gamma_L = 0.25$.

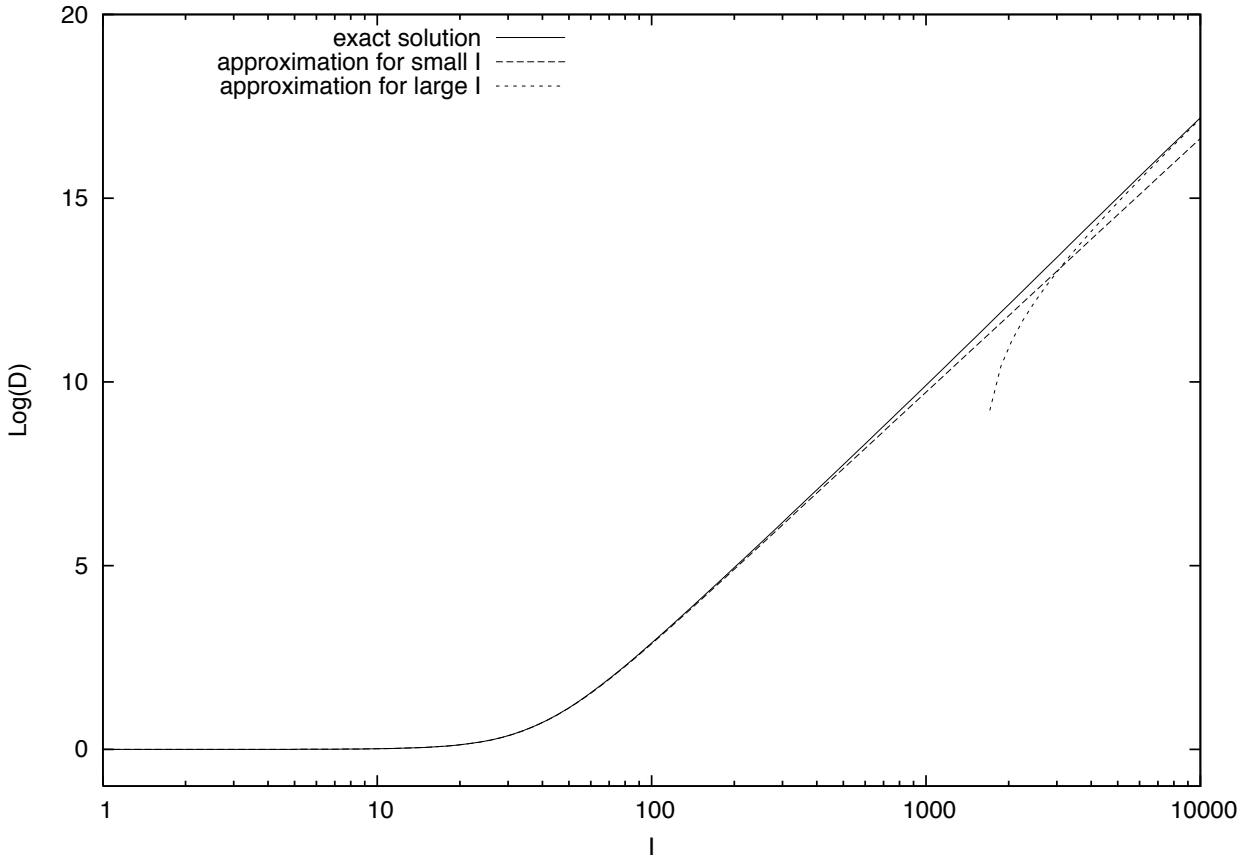

Figure 6: We confirm the approximation of D for small and large I is appropriate. $\log(D)$ obtained from exact solution and approximated D for small and large I are plotted as a function of I. The result obtained from approximated D for small I (10) is well correspond to the exact D, when I is still small. While the result obtained from approximated D for large I (11) is well correspond to the exact D, when I becomes large. These result indicates that the approximation for small and large I are appropriate. Parameters: $\gamma_E = \gamma_L = 0.1[\mathrm{nM}^{-1}\mathrm{h}^{-1}]$

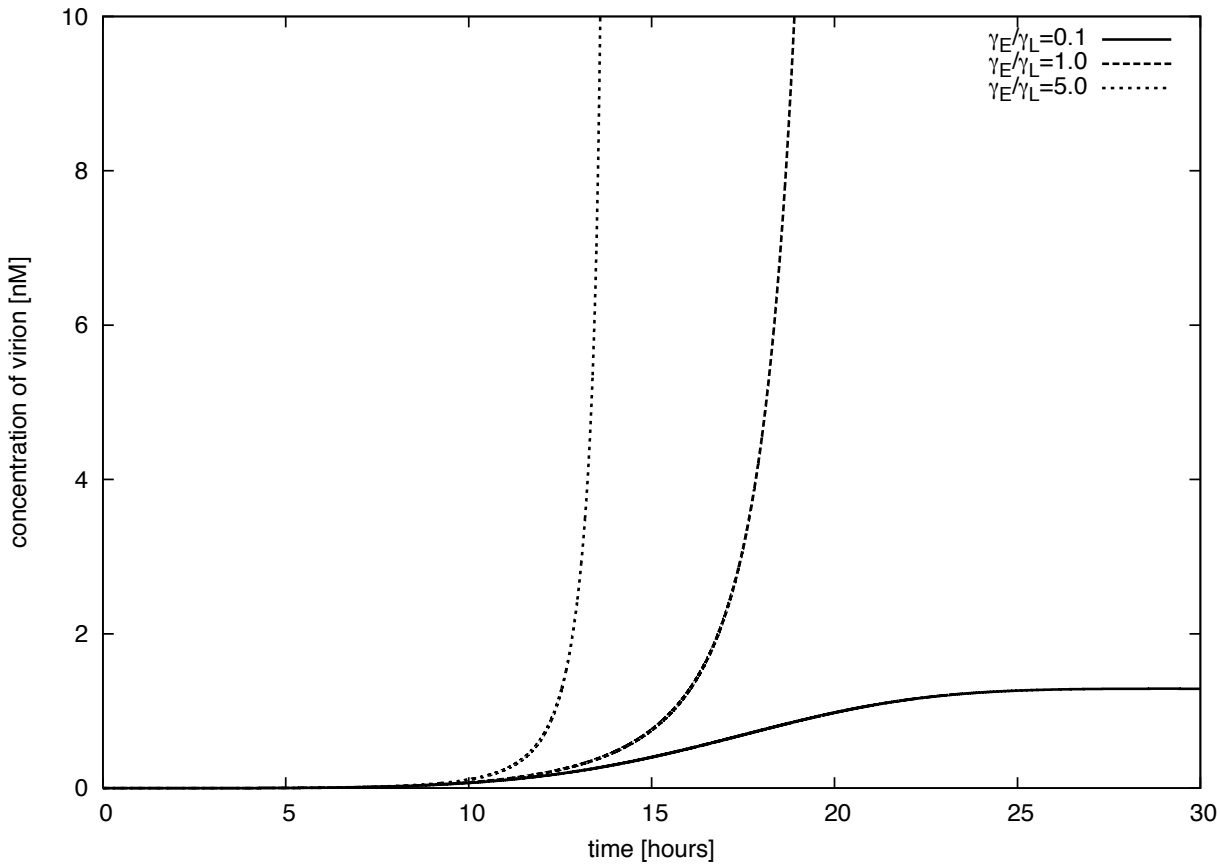

Figure 7: The time course of the concentration of virion. The time courses of the concentration of virion with various γ_E are plotted. When γ_L is fixed and γ_1 is increased from 0.01 to 0.5[nM^{-1}h^{-1}]. The reproduction speed of the virion becomes faster as γ_E becomes larger.

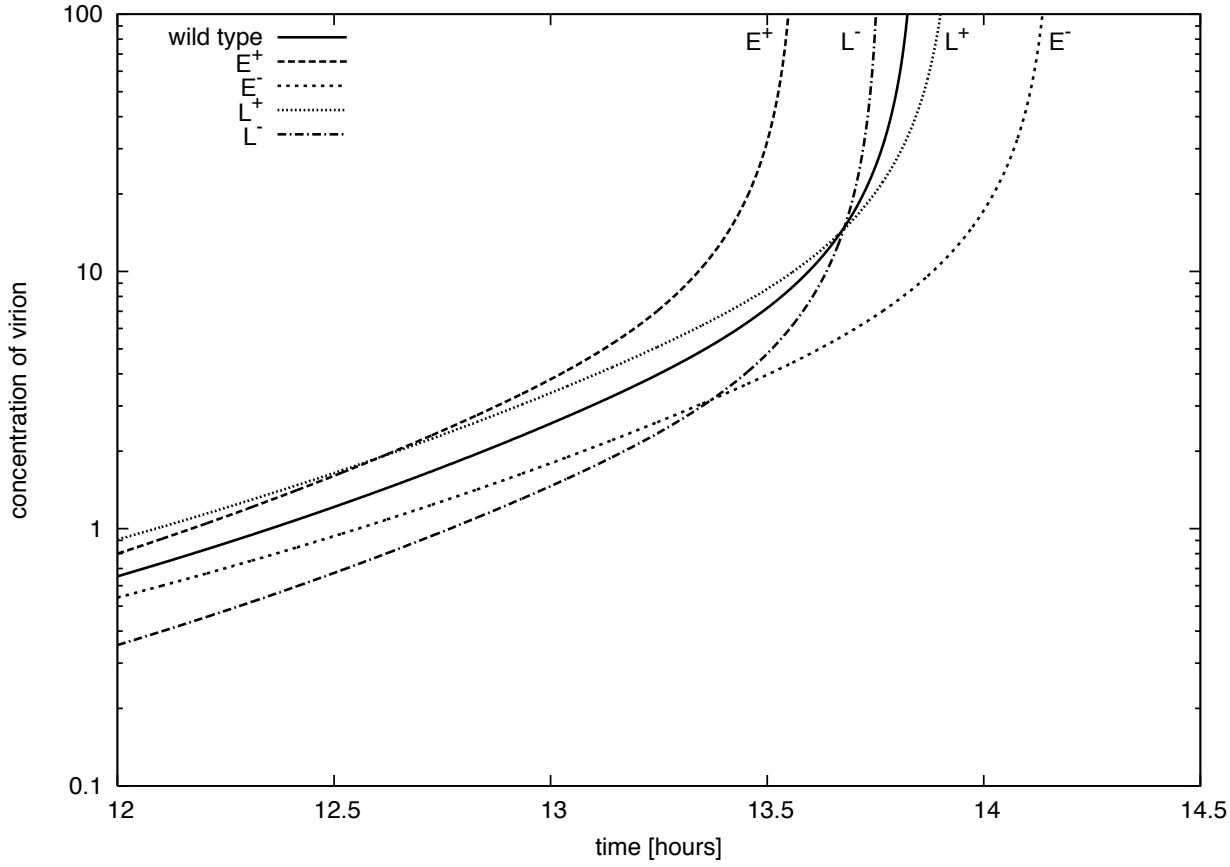

Figure 8: The mutant of which the binding affinity of the promoter is changed by the mutation. The mutant E^+ of which the binding affinity of the early gene promoter is increased by the creation of new binding site increases most rapidly in other mutants and wild type. The mutants with lower binding affinity of the early gene promoter designated by E^- and higher binding affinity of late gene promoter designated by L^+ cannot increase more rapidly than wild type. The parameters for wild type are the same in Fig 2. γ_E or γ_L of mutant are changed. $\gamma_E = 0.55$ for E^+, $\gamma_E = 0.45$ for E^-, $\gamma_L = 0.15$ for L^+ and $\gamma_L = 0.05$ for L^-.

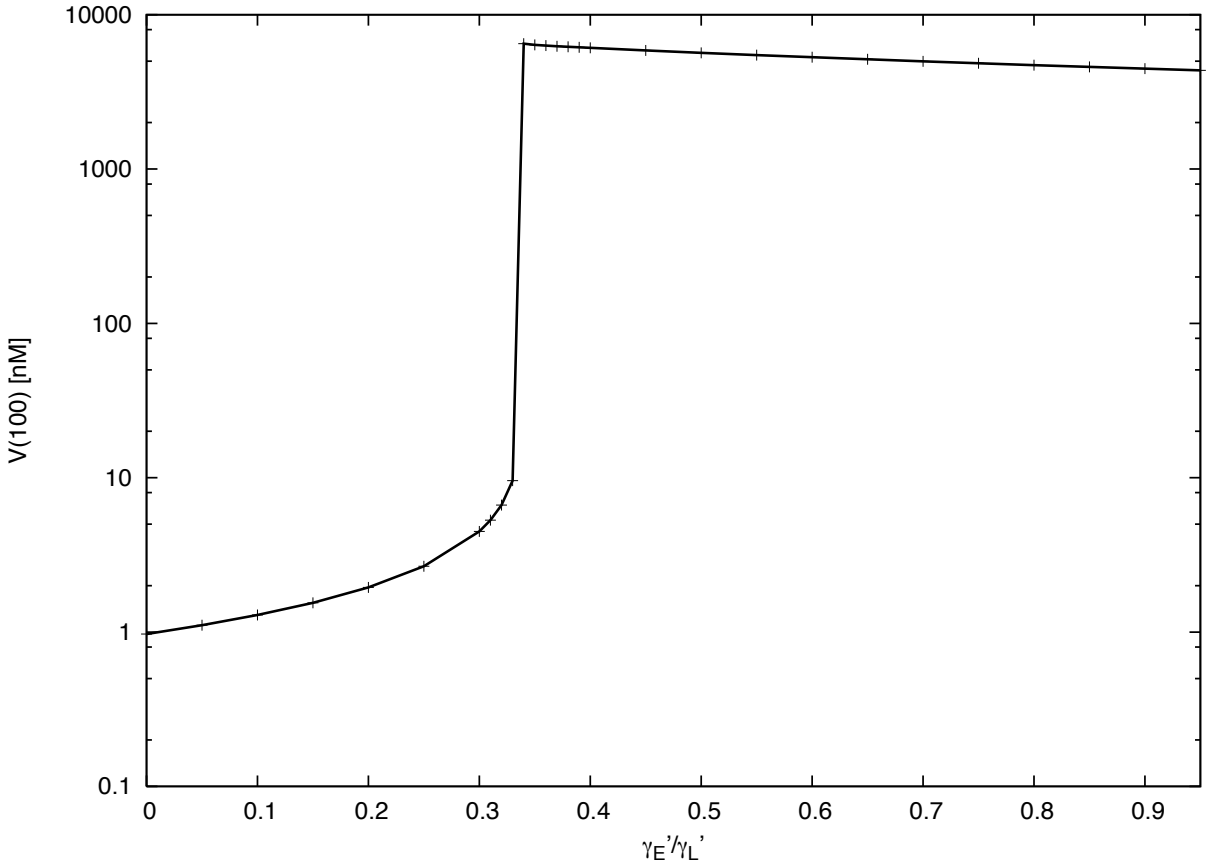

Figure 9: The final concentration of virion under the limitation of the intracellular resources. The concentration of virion is plotted against the various ratio γ'_E/γ'_L. The final concentration of virion hardly increases until γ'_E/γ'_L becomes more than the threshold level. The threshold for the explosion becomes smaller as compare to the ratio $\alpha_2/\alpha'_1 N_d(0)$ corresponding to the ratio α_2/α_1 in the model without the constraint of the resources. Parametrs:$A(0) = N_d(0) = N_r(0) = 1.0 \times 10^4$[nM]. $\alpha'_1 = 0.2 \times 10^{-4}$[nM^{-1}h^{-1}], $\alpha_2 = 0.1$[nM^{-1}h^{-1}], $\gamma'_I = 1.0 \times 10^{-4}$[nM^{-1}h^{-1}], $\gamma'_E = 0.5 \times 10^{-4}$[nM^{-1}h^{-1}], $\gamma'_L = 0.1 \times 10^{-4}$[nM^{-1}h^{-1}].

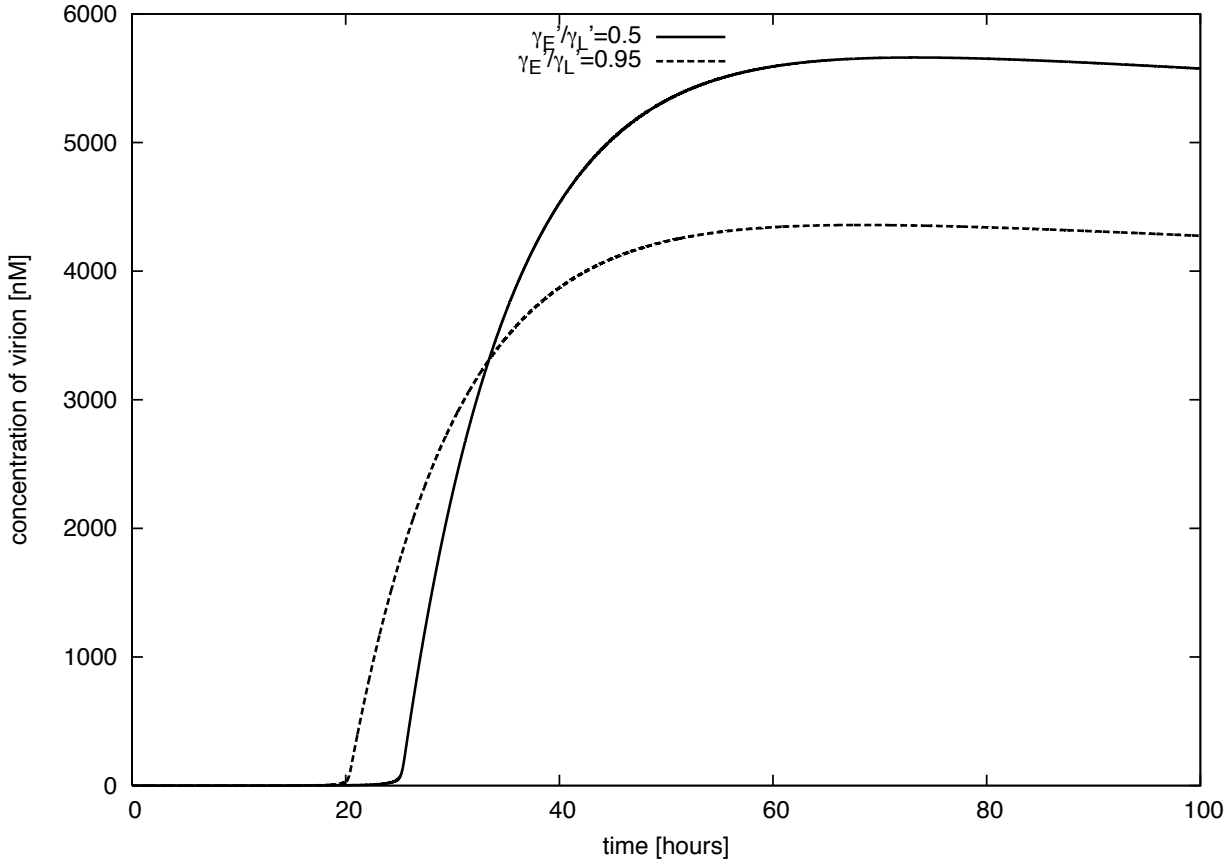

Figure 10: The time to reach the steady state. It take long time to reach the steady state, but the final concentration of the virion increases, as γ'_E/γ'_L becomes small. This result indicate that there is optimal ratio γ_E/γ_L. The optimal ratio is determined by the relationship between the speed and efficiency of complete virion production. The parameters except for γ'_E are the same in Fig 9.