International Institute for
Applied Systems Analysis
Schlossplatz 1
A-2361 Laxenburg, Austria

Tel: +43 2236 807 342
Fax: +43 2236 71313
E-mail: publications@iiasa.ac.at
Web: www.iiasa.ac.at

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A mathematical model of the intracellular replication and within host evolution of hepatitis type B virus: Understanding the long time course of chronic hepatitis

Jun Nakabayashi (nakabayashi jun@soken.ac.jp)
Akira Sasaki (sasaki_akira@soken.ac.jp)

Approved by

Ulf Dieckmann
Program Leader, EEP

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Contents

A mathematical model of the intracellular replication and within host evolution of hepatitis type B virus: Understanding the long time course of chronic hepatitis.

Jun Nakabayashi∗# and Akira Sasaki∗ † ‡

∗ Department of Evolutionary Study of Biosystems,
The Graduate University for Advanced Studies (SOKENDAI),
Hayama, Kanagawa 240-0193, Japan
Phone: +81-46-858-1580
Facsimile: +81-46-858-1542
† Evolution and Ecology Program,
International Institute for Applied Systems Analysis,
A-2361 Laxenburg, Austria ‡ PRESTO, Japan Science and Technology Agency,
4-1-8 Honcho Kawaguchi, Saitama, Japan
Corresponding author, email: nakabayashi_jun@soken.ac.jp

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Abstract

Hepatitis B virus (HBV) causes acute and chronic liver disease. Especially, chronic hepatitis is a major risk factor of liver cirrhosis and hepatocellular carcinoma. Viral kinetics of HBV observed in peripheral blood is quite different depending on the clinical course of hepatitis. But the relationship between the intracellular replication dynamics and clinical course of HBV infection is unclear. Further it is very difficult to predict the long time course of hepatitis because the nature of HBV is changed by mutation within host with high mutation rate. We investigate the intracellular replication dynamics and within host evolution of HBV by using a mathematical model. Two different intracellular replication patterns of HBV, "explosive" and "arrested", are switched depending on the viral gene expression pattern. In the explosive replication, prominent growth of HBV is observed. On the other hand, the virion production is restricted in the arrested replication. It is suggested that the arrested and explosive replication is associated with chronic hepatitis and exacerbation of hepatitis, respectively. It is shown by our evolutionary simulation that the exacerbation of hepatitis is caused by the emergence of explosive genotype of HBV from arrested genotype by mutation during chronic hepatitis. it is also shown that chronic infection without exacerbation is maintained by short waiting time for virion release and superinfection with arrested genotype. It is suggested that extension of waiting time for virion release and existence of uninfected hepatocyte in the liver may become risk factors for the exacerbation of hepatitis.

Keywords:HBV, acute and chronic hepatitis, intracellular dynamics, within

host evolution

Introduction

HBV is a major causative agent of acute and chronic hepatitis. Chronic hepatitis is a major risk factor of liver cirrhosis and hepatocellular carcinoma. The chronic hepatitis is frequently developed when the patient is infected HBV in the perinatal period or early childhood(Kao, 2007). Over 350 million people are chronically infected with HBV. More than 150 000 people die annually of hepatitis B-related liver disease (Villeneuve, 2005; Yim & Lok, 2005; Chu & Liaw, 2007). The mechanism of the persistence of HBV infection has not been fully elucidated.

The viral kinetics of HBV in chronic infection is quite different from that in acute hepatitis (Zeuzem *et al.*, 1997; Whalley *et al.*, 2001; Pawlotsky, 2003). In acute hepatitis patient, HBV DNA rapidly replicates to the order of 10^9 copies/ml. On the other hand, HBV DNA levels remain relatively stable over time at a chronic stage of infection.

It is reported that the clinical course of hepatitis is explained as the dynamics of infectious hepatocytes by using mathematical models (Payne *et al.*, 1994; Nowak *et al.*, 1996; Payne *et al.*, 1996; Tsiang *et al.*, 1999; Lau *et al.*, 2000; Columbatto *et al.*, 2006; Ciupe *et al.*, 2007*a*; Ciupe *et al.*, 2007*b*). In these papers, it is assumed that HBV virion is constantly produced with a certain rate from infected hepatocyte. The intracellular dynamics of HBV replication is not considered. It is thought that the amount of virion from single cell is varied. The viral load observed in the peripheral blood reflects total virion newly produced from each cell. The production rate of virion in the basic model

of HBV infection is interpreted as mean value of virion production from infected heaptocytes. It is considered that infected cell with large virion production is dominant as acute hepatitis. Evaluation of the virion production from single cell is necessary to understand the viral load of the hepatitis patient. Therefore it is necessary to clarify how the intracellular dynamics of HBV replication is determined to understand the clinical course of hepatitis.

We previously showed the relationship between temporal profile of viral gene expression and the intracellular dynamics of herpes simplex virus type-1 replication (Nakabayashi & Sasaki, 2009). In this paper, we construct a mathematical model of HSV-1 replication based on the molecular biological informations to investigate the intracellular dynamics of HSV-1 replication. Recently, molecular biological findings about the detailed process of HBV replication are rapidly accumulated (Moolla *et al.*, 2002; Yokosuka & Arai, 2006; Bruss, 2007). To investigate the intracellular dynamics of HBV replication, we construct a simple mathematical model based on the molecular biological findings about HBV replication.

The manifestation of hepatitis often changes during the long time course of chronic hepatitis, for example, from chronic to flare (Hunt *et al.*, 2000; Kao, 2007; Kusumoto *et al.*, 2008; Ikegami *et al.*, 2008; Cui *et al.*, 2010). Such alteration of the clinical course of hepatitis may cause within host evolution of HBV. Mutation is frequently accumulated on HBV genome with high mutation rate because of the lack of proof reading (Fang *et al.*, 2009). During the long time course of the chronic hepatitis, it is very difficult to predict the alteration of the

viral load of the chronic hepatitis patient. It is also very difficult to reproduce the evolutionary change of HBV within host by virological and cell biological experiment, because it takes long time to accumulate the mutation in HBV genome as compared with the time scale of biological experiments. Simulational approach is useful to understand the evolutionary change of HBV in an infected individual during the long time course of chronic infection. We examine whether the intracellular dynamics of HBV replication is changed by the evolutionary change of HBV within host by using our model and evolutionary simulation. In this simulation, we mainly address the long time course after the chronic hepatitis had been developed.

We first investigate the intracellular dynamics of HBV replication. It is shown by our model that two distinct replication patterns exist depending on the viral gene expression, and switching mechanism regulating the intracellular replication pattern of HBV is clarified. Next, it is shown by using the evolutionary simulation that the viral load is drastically changed during the long time course of chronic hepatitis via this switching mechanism of the replication pattern of HBV. The mutation accumulating the promoter region of HBV genome affects on the intracellular dynamics of HBV replication through the viral gene expression. Our evolutionary simulation shows the scenario that the clinical course of the hepatitis is altered by within host evolution that drastically changes the intracellular replication pattern of HBV through the alteration of viral gene expression. And finally, the mechanism robustly maintaining the chronic infection of HBV in spite of the evolutionary change of HBV is provided.

1 A mathematical model of the intracellular replication process of HBV

We construct a mathematical model of the intracellular replication of HBV based on the molecular biological informations. The diagram of HBV replication is schematically illustrated in Fig 1. HBV gene products are expressed from four different promoters. 3.5, 2.4, 2.1 and 0.7kb mRNAs are expressed from each promoter. Complete HBV virion is produced by the orchestration of these gene products. In this study, the essential component of HBV replication, 3.5 and 2.4kb mRNA, are addressed (Ueda *et al.*, 1991; Bruss & Ganem, 1991; Blum *et al.*, 1992).

The replication process of HBV has started when core particle of HBV invades into cytoplasm. Partially double stranded DNA (pdsDNA) is repaired to produce the complete closed circular DNA (cccDNA). The gap of pdsDNA of HBV genome in core particle is filled to yield a cccDNA by viral polymerase included within a core particle. The concentration of core particle and cccDNA are designated by x and y, respectively. The reaction rate constant of the transition from pdsDNA to cccDNA is designated by α_1. the chemical reaction equation of DNA repair is

$$x \quad \xrightarrow{\alpha_1} \quad y.$$

3.5kb RNA and its derivatives are related to the process of core particle replication. 3.5kb RNA is designated by R_g. The transcription of 3.5kb RNA

of HBV from cccDNA is described as follows:

$$y \quad \xrightarrow{\mu_c} \quad y + R_g$$

Here, μ_c indicates the transcription rate constant of 3.5kb RNA. The transcriptional activity of core promoter is reflected by μ_c.

A part of 3.5kb RNA is translated to viral polymerase. Another part of 3.5kb RNA is translated to core protein. The viral polymerase and core protein are designated by p and c, respectively. 3.5kb RNA and polymerase are coated by core protein to produce the core particle. Reaction rate constant of translation of core protein and polymerase are designated by β_c and β_p, respectively. 3.5kb RNA is reversely transcribe to genome DNA by viral polymerase. The RNA-protein complex composed of 3.5kb RNA and polymerase (RNP) is designated by z. The reaction rate constant of interaction between 3.5kb RNA and polymerase for reverse transcription is designated by γ_1. Replicated HBV genome DNA with polymerase is packed by core protein to produce the core particle. The reaction rate constant of the interaction between core protein and DNA-polymerase complex to produce core particle is designated by γ_2. The chemical reaction equation of core particle production is

$$R_g \xrightarrow{\beta_p} p$$

$$R_g \xrightarrow{\beta_c} c$$

$$R_g + p \xrightarrow{\gamma_1} z$$

$$z + c \xrightarrow{\gamma_2} x.$$

2.1kb and 2.4kb mRNA are translated to the surface proteins of HBV par-

ticle. The core particle is enveloped by large (LS), middle (MS) and major S (SS) proteins. These three surface proteins of HBV derived from 2.4kb mRNA is necessary for the production of infectious particle.(Ueda *et al.*, 1991). There fore 2.4kb mRNA is addressed as the component of virion in our model. 2.4kb mRNA and its gene product, surface protein, contribute to encapsidation to produce complete virion. 2.4kb mRNA and the surface protein are designated by R_s and S, respectively. The transcription rate constant of 2.4kb mRNA and the translation rate of surface protein are designated by μ_s and β_s, respectively. The promoter activity of S-promoter is reflected by μ_s. The chemical reaction equation of 2.4kb mRNA and the surface protein is described as follows:

$$\begin{aligned} y &\xrightarrow{\mu_s} y + R_s \\ R_s &\xrightarrow{\beta_s} S \end{aligned}$$

The core particle is coated by the surface protein to produce the complete virion. The reaction rate constant between the surface protein and core particle to produce complete virion is described as follows:

$$x + S \xrightarrow{\alpha_2} v$$

Each component degrades with its degradation rate.

The time change of the viral genes and gene products is given by above

mentioned chemical reaction equations.

$$
\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x - \alpha_2 S x + \gamma_2 z c - \delta_x x \\
\frac{dy}{dt} &= \alpha_1 x - \delta_y y \\
\frac{dR_g}{dt} &= \mu_c y - \gamma_1 R_g p - \delta_{R_g} R_g \\
\frac{dp}{dt} &= \beta_p R_g - \gamma_1 R_g p - \delta_p p \\
\frac{dz}{dt} &= \gamma_1 R_g p - \gamma_2 z c - \delta_z z \\
\frac{dc}{dt} &= \beta_c R_g - \gamma_2 z c - \delta_c c \\
\frac{dR_s}{dt} &= \mu_s y - \delta_{R_s} R_s \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 S x - \delta_S S \\
\frac{dv}{dt} &= \alpha_2 S x - \delta_v v
\end{aligned} \tag{1}
$$

Notations are summarized in Table 1 The dynamics of HBV replication is investigated by using this model.

1.1 The intracellular replication pattern of HBV.

Time course of HBV replication is calculated from our model as shown in Fig 2. First, cccDNA is repaired from pdsDNA in core particle. The cccDNA increases just after the infection at time 0. The viral gene products, 3.5kb RNA and 2.4kb mRNA are expressed from cccDNA with transcription rate μ_c and μ_s, respectively. The core particle is replicated by using pregenome RNA included in 3.5kb RNA as a template by viral polymerase and core protein. The core particle increases following cccDNA. And last, HBV virion is newly produced by the encapsidation of core particle by the surface protein which is translated

from 2.4kb mRNA.

The viral particle designated by v in our model represents the intracellular virion of HBV. Though the intracellular dynamics addressed in this study cannot be directly compared with the virion in the basic model of HBV infection shown in the previous studies(Payne *et al.*, 1994; Nowak *et al.*, 1996; Payne *et al.*, 1996; Tsiang *et al.*, 1999; Lau *et al.*, 2000; Ciupe *et al.*, 2007*a*; Ciupe *et al.*, 2007*b*), the amount of virion calculated from our model is related to the production rate of virion that is assumed as constant in these studies. Some parameters in our model such as association rate between the core particle and surface protein are difficult to directly measure. The parameters are estimated that v obtained from our model is compared with the production rate of virion in the basic model of HBV infection. For example, the virion production during adefovil dipivoxil therapy is estimated from 8.3×10^{10} to 9.2×10^{12} copies/day by Tsiang *et al.* (Tsiang *et al.*, 1999). The virion production about 24 hrs obtained from our model becomes 4.0×10^{12} as shown in Fig 2A. This result indicates that the intracellular replication process of HBV is reproduced by our model.

The parameter dependence for maximum value of virion production is investigated and summarized in Table 1. When the parameters except for the selected one are fixed, the maximum value of v are obtained. A part of these result is shown in Fig 3. The number of virion v monotonically increases or decreases when the selected parameter is changed within the exploration range, except for the degradation rate of polymerase, δ_p. The parameter increasing or decreasing the maximum value of the virion production as this selected pa-

rameter increases is indicated by + or −, respectively in Table 1. There are some parameters paradoxically affecting on the virion production. For example, the virion production decreases as the reaction rate constant for the virion production, α_2, increases. This result indicates that the behavior of the entire system of HBV replication is complicated. It is important to understand how intracellular dynamics of HBV replication is determined.

Among the parameters, the effect of transcription rate of 3.5kb mRNA, μ_c, for the virion production is significant. So we focus on the relationship between viral gene expression and the virion production. Replication dynamics of HBV is drastically changed depending on the viral gene expression pattern as shown in Fig 2A and B. When the expression ratio of 3.5kb RNA to 2.4kb mRNA designated by μ_c/μ_s is large, the virion continues to exponentially increase as shown in Fig 2A. This exponential replication of HBV is caused by the positive feedback loop of core particle replication. The replicated core particle is accumulated and can contribute to produce 3.5kb RNA. The core particle is further replicated by the gene products of 3.5kb RNA. The replication of the complete virion is enhanced by the effect of this positive feedback. On the other hand, the virion production is finally arrested after sufficiently long time has passed from infection when the ratio μ_c/μ_s is small as shown in Fig 2B. The core particle reaches peak and then decrease to converge to 0. The core particle is consumed to produce the complete virion. Under this expression ratio, the positive feedback cannot work because the encapsidation of core particle by the surface protein exceeds its replication. The virion is produced until cccDNA is

completely degraded. The restricted reproduction of HBV is related to HBV dynamics observed in peripheral blood of the chronic hepatitis patient. Logarithmic plot of the virion v is shown in Fig 2C. When the expression ratio μ_c/μ_s exceeds a threshold level, the virion continues to exponentially increase and finally diverges to infinite.

The maximum number of virion during the replication is shown as contour plot in Fig 4. Here we mention the continuous replication with large μ_c/μ_s and the arrested replication with small μ_c/μ_s as an "explosive" and an "arrested" replication, respectively. Bright area on the contour plot indicate the region where the explosive replication is caused.

1.2 Threshold μ_c/μ_s ratio for the explosive replication.

To analytically obtain the threshold ratio μ_c/μ_s for the explosive replication, we simplify our model. First of all, degradation of viral gene product is considered. Viral replication cannot proceed if the degradation of viral gene product rapidly proceeds as compare with other reaction composed for viral replication cycle. It is naturally considered that the degradation rate constants are smaller than those of other reaction rate constants. The degradation of viral gene product

can be ignored. The model is simplified as follows:

$$\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x - \alpha_2 S x + \gamma_2 z c \\
\frac{dy}{dt} &= \alpha_1 x \\
\frac{dR_g}{dt} &= \mu_c y - \gamma_1 R_g p \\
\frac{dp}{dt} &= \beta_p R_g - \gamma_1 R_g p \\
\frac{dz}{dt} &= \gamma_1 R_g p - \gamma_2 z c \\
\frac{dc}{dt} &= \beta_c R_g - \gamma_2 z c \\
\frac{dR_s}{dt} &= \mu_s y \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 S x \\
\frac{dv}{dt} &= \alpha_2 S x
\end{aligned}$$

Next, the viral polymerase p is considered. Null cline of viral polymerase is constant, β_p/γ_1. Viral polymerase cannot be accumulate beyond this saturation level during the replication process. It is assumed that the concentration of viral polymerase quickly reaches this saturation level. This yields $p = \beta_p/\gamma_1$.

Substituting this into full kinetic system (1), the model is simplified as follows:

$$\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x - \alpha_2 S x + \gamma_2 z c \\
\frac{dy}{dt} &= \alpha_1 x \\
\frac{dR_g}{dt} &= \mu_c y - \beta_p R_g \\
\frac{dz}{dt} &= \beta_p R_g - \gamma_2 z c \\
\frac{dc}{dt} &= \beta_c R_g - \gamma_2 z c \\
\frac{dR_s}{dt} &= \mu_s y \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 S x \\
\frac{dv}{dt} &= \alpha_2 S x
\end{aligned}$$

Next, 3.5kb RNA is accumulated depending on the accumulation of cccDNA, y, from the initial condition, $R_g(0) = 0$. On $R_g - y$ phase plane, R_g increases just below the null cline $R_g = \mu_c y/\beta_p$ from the initial condition $(R_g, y) = (0, 0)$. This yield $\mu_c y = \beta_p R_g$.

$$\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x - \alpha_2 S x + \gamma_2 z c \\
\frac{dy}{dt} &= \alpha_1 x \\
\frac{dz}{dt} &= \mu_c y - \gamma_2 z c \\
\frac{dc}{dt} &= \frac{\beta_c}{\beta_p} \mu_c y - \gamma_2 z c \\
\frac{dR_s}{dt} &= \mu_s y \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 S x \\
\frac{dv}{dt} &= \alpha_2 S x
\end{aligned}$$

Here, it is assumed that the translation rate of the core protein is same as that

of the viral polymerase, time change of the core protein becomes same as that of RNP. As well as R_g, core protein and RNP is accumulated from initial condition $c(0) = z(0) = 0$ depending on cccDNA, y. Therefore c and z increase just below their null cline on $cz - y$ phase plane. This yields $\gamma_2 zc = \mu_c y$. The model is simplified as follows:

$$\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x - \alpha_2 Sx + \mu_c y \\
\frac{dy}{dt} &= \alpha_1 x \\
\frac{dR_s}{dt} &= \mu_s y \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 Sx \\
\frac{dv}{dt} &= \alpha_2 Sx
\end{aligned}$$

Finally, the surface protein is considered. As well as 3.5kb RNA and its gene products, S increases from $S(0) = 0$ depending on R_s. This yields $\beta_s R_s = \alpha_2 Sx$. As a result, the full kinetics system (1) can be simplified as follows:

$$\begin{aligned}
\frac{dx}{dt} &= -\alpha_1 x + \mu_c y - \beta_s R_s \\
\frac{dy}{dt} &= \alpha_1 x \\
\frac{dR_s}{dt} &= \mu_s y
\end{aligned} \tag{2}$$

The dynamics of this simplified system is determined by x. From (2), homogenous equation of x is

$$\frac{d^3x}{dt^3} + \alpha_1 \frac{d^2x}{dt^2} - \alpha_1 \mu_c \frac{dx}{dt} + \alpha_1 \beta_s \mu_s x = 0. \tag{3}$$

Characteristic equation of (3) is

$$\lambda^3 + \alpha_1 \lambda^2 - \alpha_1 \mu_c \lambda + \alpha_1 \mu_s \beta_s = 0. \tag{4}$$

If (4) has three real solution, the time dependent solution of x is obtained as exponential function as follows:

$$C_1 \exp[\lambda_1 t] + C_2 \exp[\lambda_2 t] + C_3 \exp[\lambda_3 t] \tag{5}$$

Here, λ_1, λ_2 and λ_3 are real solution of (4). And C_1 , C_2 and C_3 are constants. The virion v diverges to infinite in this case. We can obtained the threshold condition for the explosive replication of HBV when characteristic equation has three real solution,

$$\alpha_1^4\mu_c^2 + 4\alpha_1^3\mu_c^3 - 4\alpha_1^4\beta_s\mu_s - 18\alpha_1^3\beta_s\mu_c\mu_s - 27\alpha_1^2\beta_s^2\mu_s^2 > 0. \tag{6}$$

The threshold μ_s is expressed from (6) as

$$\mu_s < \frac{2\sqrt{\alpha_1\beta_s^2(\alpha_1 + 3\mu_c)^3} - \alpha_1\beta_s(2\alpha_1 + 9\mu_c)}{27\beta_s^2}. \tag{7}$$

Where α_1 and β_s indicate transition rate constant from core particle to cccDNA and translation rate constant of the surface protein, respectively. The threshold μ_c and μ_s with fixed α_1 and β_s are indicated by red line in Fig 4A. The threshold (7) analytically obtained from simplified model (2) well agree with the threshold from numerical calculation of full kinetics model (1). Time course of the core particle obtained from simplified model is compared with that from full kinetics model as shown in Fig 4B and C. The core particle x obtained from simplified model proceeds to that from the full kinetics model. This gap is caused by the effect ignoring the degradation term. But the behavior of the core particle after sufficiently long time has passed, whether becomes 0 or diverges to infinite is conserved in both models. As a result, the threshold (7) obtained

from simplified model (2) agree with that from the full kinetics model as shown in Fig 4A.

Though this condition is seemingly complicated, it is easy to intuitionally understand the mechanism determining the replication pattern. 3.5kb RNA including pre-genome RNA, mRNA of polymerase and core protein contributes to production of core particle. Increasing the transcription rate of 3.5kb RNA, which means large μ_c in our model, enhances the replication of core particle. Viral gene expression from cccDNA repaired from pdsDNA in core particle is also enhanced. The virion is explosively increased by the effect of the positive feedback loop of core particle replication, illustrated by upper half of the diagram of replication process in Fig 1. On the other hand, increasing 2.4kb mRNA expression decreases the core particle by its encapsidation to produce the complete virion. The core particle is exhausted by excessive expression of surface protein. The cccDNA converges to a certain positive value because transition from core particle to cccDNA that is source of viral gene expression is stopped. In the simplified model, HBV is constantly reproduced from cccDNA converging to a certain positive concentration because degradation terms are ignored. The replication of HBV is arrested in full kinetics model when all cccDNA is completely degraded. As a result, replication pattern of HBV is drastically changed by little difference of the expression ratio, μ_c/μ_s.

2 The effect of within host evolution of HBV for the clinical course of hepatitis.

As mentioned above, the mechanism switching the intracellular replication pattern of HBV is understood. The virion production is drastically changed by slight difference of the expression ratio μ_c/μ_s around the threshold level. The intracellular dynamics of HBV replication may be changed when the mutation in the promoter region affecting on the promoter activity is accumulated. The phenotype of HBV such as viral gene expression pattern is changed by the mutation (Buckwold *et al.*, 1996; Hasegawa *et al.*, 1994; Moriyama *et al.*, 1996; Günther *et al.*, 1998; Jammeh *et al.*, 2008). Therefore we next consider the effect of evolutionary change of HBV within host for clinical course of hepatitis. The contour plot of the concentration of virion is considered as a fitness landscape of HBV with various viral gene expression patterns, because the amount of newly produced virion indicates a fitness of HBV. When the expression rate of viral gene is changed as a quantitative phenotype by the mutation on the promoter region, HBV with μ_c/μ_s ratio maximizing the virion production is selected. We confirm weather explosive genotype can evolve from the arrested type of HBV by using a evolutionary simulation. HBV with various viral gene transcription rate are generated by drawing the random number for μ_c and μ_s. The amount of virion of j-th particle of HBV at n-th generation is indicated by $v_n^{(j)}$. Each HBV particle has a slightly different parameter set $(\mu_{c,n}^{(j)}, \mu_{s,n}^{(j)})$. It is assumed that each HBV replicates its copy in single infected cell. The amount of each virion is calculated according to our model (1). Here, time τ is defined as

waiting time from infection until newly produced virion is released. After HBV replicates its copy in infected cell during time τ, virion is released to expand the infection to uninfected cell. It is assumed that number of infected cell with j-th HBV particle depends on the relative amount of virion, $v_n^{(j)}(\tau)/\sum(v_n^{(j)}(\tau))$. This means that HBV with transcription rates producing larger amount of virion increases its frequency. HBV particles are resampled that the frequency of HBV with parameter, $(\mu_{c,n}^{(k_j)}, \mu_{s,n}^{(k_j)})$, depends on relative amount of virion. To repeat the infection, the frequency of HBV with a parameter that enlarge the amount of virion at time τ increases. The parameter is changed by the mutation as generation n increases. The mutation is reproduced by adding the random number to μ_c and μ_s respectively in the evolutionary simulation. Here, one generation in this simulation is defined as time when J mutants with different transcription rates are accumulated. The procedure of the simulation is summarized as follows:

(1) Set HBV particle with various $(\mu_{c,n}^{(j)}, \mu_{s,n}^{(j)})$

(2) For $n = 1, \cdots, N$

(3) For $j = 1, \cdots, J$

(4) Calculate $v_n^{(j)}(\tau) = F(\tau, \mu_{c,n}^{(j)}, \mu_{s,n}^{(j)})$

(5) Resample HBV particle $\text{Prob}(k = j) \sim v_n^{(j)}(\tau) / \sum v_n^{(j)}$

$(k = 1, \cdots, J)$

(6) End for j

(7) Set $\mu_{c,n}^{(j)} + \omega, \mu_{s,n}^{(j)} + \omega$

ω : random noise $\omega \sim \text{Normal}(\bar{\omega}, \sigma)$

(8) End for n

It is assumed that superinfection is not occurred. We use parameters: $J = 1000$, $\bar{\omega} = 0$, $\sigma = 0.001$. A sample path of the evolutionary simulation is shown in Fig 5. Simulation is repeated to generation $N = 5000$ with $\tau = 300$ [min]. The mean value of μ_c, μ_s and $v(\tau)$ are plotted. The frequency of HBV with large μ_c or small μ_s increases along with the generation. The mean amount of virion, indicated by the vertical axis of right hand side in Fig 5, also gradually increases as mean of μ_c (μ_s) increases (decreases). When μ_c/μ_s ratio exceeds a threshold level, the amount of virion becomes prominently large. This result suggests that the exacerbation of hepatitis is caused by the evolutionary change of HBV emerging the explosive genotype during the long time course. The evolutionary

change of HBV affects the clinical manifestation during the long time course. It takes about 4000 generations in this simulation to explosively increase the virion production. If the exacerbation of hepatitis occurs 20 years after infection, it is estimated that 1000 mutants with different transcription rate of viral gene accumulates in 1.825 days.

2.1 The condition maintaining the chronic infection without exacerbation.

In this framework of HBV evolution, the expression rate of 3.5kb RNA always increases by mutation on the promoter region during the clinical course of hepatitis because HBV virion with large μ_c/μ_s increases. Viral gene expression pattern always evolves toward right-bottom side on the graph in Fig 4. The emergence of the explosive genotype is inevitable. But there is a patient who passes the long time course without exacerbation. Therefore we next investigate the condition for maintaining the chronic infection to prevent the emergence of the explosive genotype.

The effect of waiting time τ for HBV virion release from cell for within host evolution is investigated. Contour plot of HBV virion v with various τ is shown in Fig 6A and B. τ is 20 [min] and 300 [min] for Fig 6A and B, respectively. The threshold μ_c/μ_s ratio analytically obtained is indicated by red line. These graphs indicate how fitness landscape of HBV with various viral gene expression pattern is changed depending on τ. From these graphs, we understand which replication pattern of HBV can evolve. When waiting time for virion release is small (Fig 6A), the concentration of virion increases as μ_s becomes large. HBV

with μ_c/μ_s ratio that maximizes the virion production evolves to right-top side on the graph in Fig 6A. The optimum μ_c/μ_s ratio is beyond the threshold for the explosive replication. In this case, arrested type of HBV can evolve and the virion production remains low level. A sample path of evolutionary simulation with small τ ($\tau = 20$ [min]) is shown in Fig 7. This represents the chronic hepatitis without exacerbation. Transcription rate of 2.4kb mRNA becomes large and the number of virion remain low. This result indicate that the chronic hepatitis without exacerbation is maintained when waiting time for HBV release is short.

2.2 Trade-off between the production speed and the final concentration of virion.

The mechanism that arrested type of HBV can evolve when waiting time for the virion release becomes small is considered. In the early phase of the replication, the virion arise faster when μ_s is large because the replicated core particle is rapidly encapsidated by the surface protein. After a while, the virion production with large μ_c/μ_s exceeds to that with small μ_c/μ_s by the effect of the positive feedback as shown in Fig 8. This result indicates that there is a trade-off between the initial speed of the virion production and the final production of virion. Arrested genotype can evolve in spite of the restricted production of virion by the fast production of virion when the waiting time for virion release is small. As waiting time for the virion release becomes large, HBV with large μ_c/μ_s ratio becomes advantageous. When the optimum expression ratio is below the threshold for the explosive replication as shown in Fig 6B, the explosive genotype

can evolve.

3 Effect of the superinfection for within host evolution of HBV.

3.1 A model of superinfection in single cell

In the previous sections, it is assumed that the superinfection of HBV is ignored. Our model is expanded to include the superinfection of HBV. Because genetically distinct variants of HBV coexist in an infected individual as quasispecies(Pawlotsky, 2005; Sheldon *et al.*, 2006). It is possible that the distinct variants simultaneously infect in single cell. For the simplicity, it is assumed there are two genotypes of HBV, arrested and explosive genotype. The genotype of HBV is determined by its structure of promoter region. HBV with promoter which satisfies the threshold condition for the explosive replication is identified as a explosive genotype. One cell is simultaneously infected by both explosive and arrested genotype. Core particle (x), cccDNA (y), pregenome RNA (R_g) and intermediate RNA polymerase complex (z) are distinguished by the genotype. The subscript A and E indicate arrested and explosive genotype, respectively. Core protein, polymerase and the surface protein from both genotypes are not distinguished because there is no difference of the coding sequence of these gene products. Here, we examine the effect of the superinfection of HBV for within host evolution of HBV. It is consider that one cell is simultaneously infected by both explosive and arrested types. The diagram of HBV replication

under the super infection is shown in Fig 10.

$$
\begin{aligned}
\frac{dx_A}{dt} &= -\alpha_1 x_A - \alpha_2 S x_A + (1-\lambda)\gamma_2 z_A c + \lambda\gamma_2 z_E c - \delta_x x_A \\
\frac{dy_A}{dt} &= \alpha_1 x_A - \delta_y y_A \\
\frac{dR_{gA}}{dt} &= \mu_{cA} y_A - \gamma_1 R_{gA} p - \delta_{R_g} R_{gA} \\
\frac{dz_A}{dt} &= \gamma_1 R_{gA} p - \gamma_2 z_A c - \delta_z z_A \\
\frac{dx_E}{dt} &= -\alpha_1 x_E - \alpha_2 S x_E + (1-\lambda)\gamma_2 z_E c + \lambda\gamma_2 z_A c - \delta_x x_E \\
\frac{dy_E}{dt} &= \alpha_1 x_E - \delta_y y_E \\
\frac{dR_{gE}}{dt} &= \mu_{cE} y_E - \gamma_1 R_{gE} p - \delta_{R_g} R_{gE} \\
\frac{dz_E}{dt} &= \gamma_1 R_{gE} p - \gamma_2 z_E c - \delta_z z_E \\
\frac{dp}{dt} &= \beta_p (R_{gA} + R_{gE}) - \gamma_1 (R_{gA} + R_{gE}) p - \delta_p p \\
\frac{dc}{dt} &= \beta_c (R_{gA} + R_{gE}) - \gamma_2 (z_A + z_E) c - \delta_c c \\
\frac{dR_s}{dt} &= \mu_{sA} y_A + \mu_{sE} y_E - \delta_{R_s} R_s \\
\frac{dS}{dt} &= \beta_s R_s - \alpha_2 (x_A + x_E) S - \delta_S S \\
\frac{dv_A}{dt} &= \alpha_2 S x_A - \delta_v v_A \\
\frac{dv_E}{dt} &= \alpha_2 S x_E - \delta_v v_E
\end{aligned} \tag{8}
$$

Here, λ indicate the mutation rate of the promoter. The transition between two genotypes is constantly occurred with mutation rate λ ($\lambda = 1.0 \times 10^{-4}$ in the simulation).

Time course of the virion production obtained from expanded model is shown in Fig 10A and B. In Fig 10A, the virion of both genotype converge to a certain constant. In spite that the expression ratio μ_{cE}/μ_{sE} which is same as the

value shown by the dashed line in Fig 2C ($\mu_{cE}/\mu_{sE} = 9$) satisfies the threshold condition for the explosive replication, v_E cannot diverge to infinite. This result indicates that superinfection with arrested type of HBV prevents the explosive replication of HBV. This prevention is caused by the excessive expression of the surface protein from arrested type. Core particle of explosive genotype is consumed by the interaction with the surface protein from arrested type. As a result, the effect of the positive feedback is inhibited.

When the expression of 3.5kb mRNA is extremely excess the threshold for the explosive replication ($\mu_{cE}/\mu_{sE} = 15$), v_E can finally diverge to infinite under the superinfection with arrested genotype as shown in Fig 10B. And v_A also continues to increase.

3.2 Evolutionary simulation with superinfection

Evolutionary simulation is also expand to include the superinfection. Thought the procedure of the evolutionary simulation is same as shown in previous section, it is assumed that all hepatocytes are already infected by arrested type ($\mu_{cA/}\mu_{sA} = 0.5$) in the expanded simulation. This μ_{cA}/μ_{sA} ratio of arrested type is fixed. The mutant with various μ_{cE}/μ_{sE} ratio invades into hepatocyte infected with arrested type. The number of particle of mutant virion with various μ_{cE}/μ_{sE} is calculated by using the model of superinfection (8). The frequency of the mutant is evaluated by the relative amount of the virion. Through the evolutionary simulation, mutant with large μ_{cE} and small μ_{sE} increase its frequency as well as shown in Fig 5, and the virion production then gradually increases. For the prominent increase of virion production, extremely large

μ_{cE}/μ_{sE} ratio is necessary. As compared with a sample path in Fig 5, μ_{cE} becomes larger (over $0.1[\text{min}^{-1}]$). The mutant almost lacking the surface protein expression emerges. As predicted from the model of superinfection in single cell, the explosive replication finally occurs when μ_{cE}/μ_{sE} ratio greatly exceed the threshold condition for the explosive type (7).

Discussion

Our model clarified the relationship between the intracellular dynamics of HBV replication and the clinical course of the hepatitis. It is shown by our model that a little difference of expression ratio of 3.5kb RNA to 2.4kb mRNA can switch the intracellular replication pattern of HBV. Adeno-associated virus Rep78 protein specifically binds to HBV core promoter(Liu *et al.*, 2009). And the replication of HBV is inhibitied through the inhibition of transcriptional activity of HBV core promoter. This result supports our result that the reduction of core promoter activity that means small μ_c in our model reduces the virion production.

Core promoter region is focused by the relation to the virion reproduction. Mutation of HBV on the precore/core promoter region is intensively studied as a viral factor affecting the severity of liver disease (Günther, 2006; Ozasa *et al.*, 2006; Du *et al.*, 2007; Tong *et al.*, 2007). Naturally occurred mutation of HBV in promoter region is frequently observed. But the effect of the mutation on HBV replication and clinical course is not understood as a simple context. For example, it is reported that frequently observed mutation in promoter region, A1762T

and G1764A, associates with both chronic and acute hepatitis. Ambiguity of the effect of mutation on promoter region may relate that the replication dynamics of HBV is sensitively affected by the expression ratio around the threshold condition. It is revealed that A1762T/G1764A double mutation decreases the promoter activity by reporter assay (Dong *et al.*, 2008). If A1762T/G1764A mutation associates with chronic hepatitis by decreasing virion production with low 3.5kb RNA expression, our model well explain the mechanism decreasing the virion production.

Among these mutation in core-promoter region, we focus on the mutation creating a new binding site to HNF-1. It is reported that the mutations creating the new HNF-1 binding site in core promoter correlates with the severe liver disease such as fulminant hepatitis (Günther *et al.*, 1996; Pult *et al.*, 1997; Baumert *et al.*, 1998; Li *et al.*, 1999; Fujiwara *et al.*, 2005). These mutations increase the core promoter activity, core protein synthesis and virion replication. The exacerbation of the hepatitis caused by emerging this type of mutation during the long clinical course of the chronic hepatitis is well explained by our scenario shown by the evolutionary simulation. The increase of core-promoter activity by new HNF-1 binding site is correspond with the large μ_c in our model. The expression of 3.5kb RNA is increased by the new HNF-1 binding site, and the production of virion prominently then increases by the effect of the positive feedback when μ_c/μ_s ratio exceeds a threshold level. Viral load is prominently increases when the hepatocytes in the patient's liver are dominantly infected by the explosive mutant. Our model provides the analytical background of the

relationship between the mutation creating the new HNF-1 binding site and sever liver disease with high viral load.

The exacerbation of hepatitis by activation of core promoter cannot always occur. Which genotype of HBV, explosive or arrested, becomes advantageous depends on waiting time for the release of newly produced HBV virion to expand the infection to the uninfected cell. There is a trade-off between the intial speed of the virion production and the final production of HBV virion. When waiting time for the virion release is sufficiently long, explosive genotype becomes advantageous to take time to explosively replicate the virion in infected cell. Whether the waiting time for virion release is sufficient that explosive replication becomes advantageous is evaluated by the contour plot of virion as shown in Fig 4 A and 6. When the bright region where the virion production is large is beyond the line of the threshold (7), waiting time is sufficiently long. Under these condition, it is possible that the explosive genotype can evolve. It is suggested by our model that waiting time for the release of HBV virion is an important factor affecting the clinical course of HBV infection. HBV virion is secreted through the exocytosis from the infected cell(Radtke *et al.*, 2006; Patient *et al.*, 2009). For the virion release, newly replicated HBV virion is moved from nucleus to cytoplasm by using the intracellular mechanism regulating the migration between subcellular sites. It is reported that X oncoprotein of HBV (HBx) interact with Crm1/Ran GTPase which function as a cargo for nuclear export(Forgus *et al.*, 2003; Yun *et al.*, 2004). The interaction between host cell and HBV that modifies the subcellular movement may affect on waiting time

for the release of HBV virion from cell. It is speculated by our model that the prolongation of waiting time for the release of HBV virion may become risk factor of the exacerbation of hepatitis.

Finally, the effect of the superinfection is considered. When one cell is infected by both the explosive and arrested genotype, the threshold condition for the explosive replication analytically obtained from simplified model (2) becomes insufficient. The excessive expression of the surface protein from the arrested genotype prevents the positive feedback effect of the explosive genotype. This result indicates that superinfection inhibits the exacerbation of hepatitis from chronic infection and contributes to maintenance of chronic infection. This inhibition of the explosive replication by excessive expression of the surface protein from arrested genotype is also shown by the evolutionary simulation in Fig 11. For the explosive replication of virion, the additional increase of μ_{cE}/μ_{sE} ratio is necessary exceeding the threshold level (7). This result indicates that, superinfection is one of the candidate of the mechanism maintaining the chronic infection of HBV without exacerbation in addition to the short waiting time for HBV release from cell. Once the arrested type of HBV occupies hepatocytes in the liver, the explosive replication of HBV is rarely caused. The explosive type can prominently replicate its copy when uninfected hepatocyte remain to solely infected with the explosive type by chance. It is also suggested that the existence of the uninfected hepatocytes may become risk factor of the exacerbation during the chronic hepatitis.

Because the intrinsic factor of HBV affecting the intracellular replication of

HBV is focused in this study, there are some factors that are not included in our model but affect the clinical course of hepatitis. In the patient, the long time course of HBV infection is affected by the complicated interaction between host and pathogen. Clinical manifestation of hepatitis sometimes changes during the lifetime long course according to the extrinsic stimuli such as anti-viral drug, chemotherapeutics and immunosuppressant (Günther *et al.*, 1996; Alexopoulou *et al.*, 2006; Tacke *et al.*, 2004; Uzun *et al.*, 2006; Heipertz *et al.*, 2007). Especially, host immune plays pivotal role in determining the clinical course of HBV infection. In this study, variety and a spatial distribution of cell are also not considered. The intracellular replication and within host evolution of HBV under a spatially uniform condition is investigated. Recently, the spatial dispersion of HBV infection in liver is modeled (Wang & Wang, 2007). Not only intrinsic factor of HBV such as viral gene expression pattern and waiting time for HBV secretion but also the host factor such as immune response and the structure of the liver are totally considered to understand the clinical course of hepatitis.

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Notation	Description	effect for virion production
x	core particle (pdsDNA)	
y	cccDNA	
R_g	3.5kb RNA	
c	core protein	
p	polymerase	
z	pregenome-polymerase complex (RNP)	
R_s	2.4kb mRNA	
s	surface protein	
v	virion	
α_1	reaction rate of DNA repair of pdsDNA	+
α_2	production rate of virion	-
μ_c	transcription rate of 3.5kb RNA	+
μ_s	transcription rate of 2.4kb mRNA	-
β_c	translation rate of core protein	+
β_p	translation rate of polymerase	-
β_s	translation rate of surface protein	-
γ_1	interaction rate between pregenome and polymerase	-
γ_2	production rate of core particle	+
δ_x	degradation rate of core particle	-
δ_y	degradation rate of core cccDNA	-
δ_{R_g}	degradation rate of 3.5kb RNA	-
δ_{R_s}	degradation rate of 2.4kb mRNA	+
δ_c	degradation rate of core protein	-
δ_p	degradation rate of polymerase	convex
δ_s	degradation rate of surface protein	+
δ_z	degradation rate of pregenome-polymerase complex	-
δ_v	degradation rate of virion	-
τ	waiting time for HBV release from cell	

Table 1: List of the variables and parameters of the model.

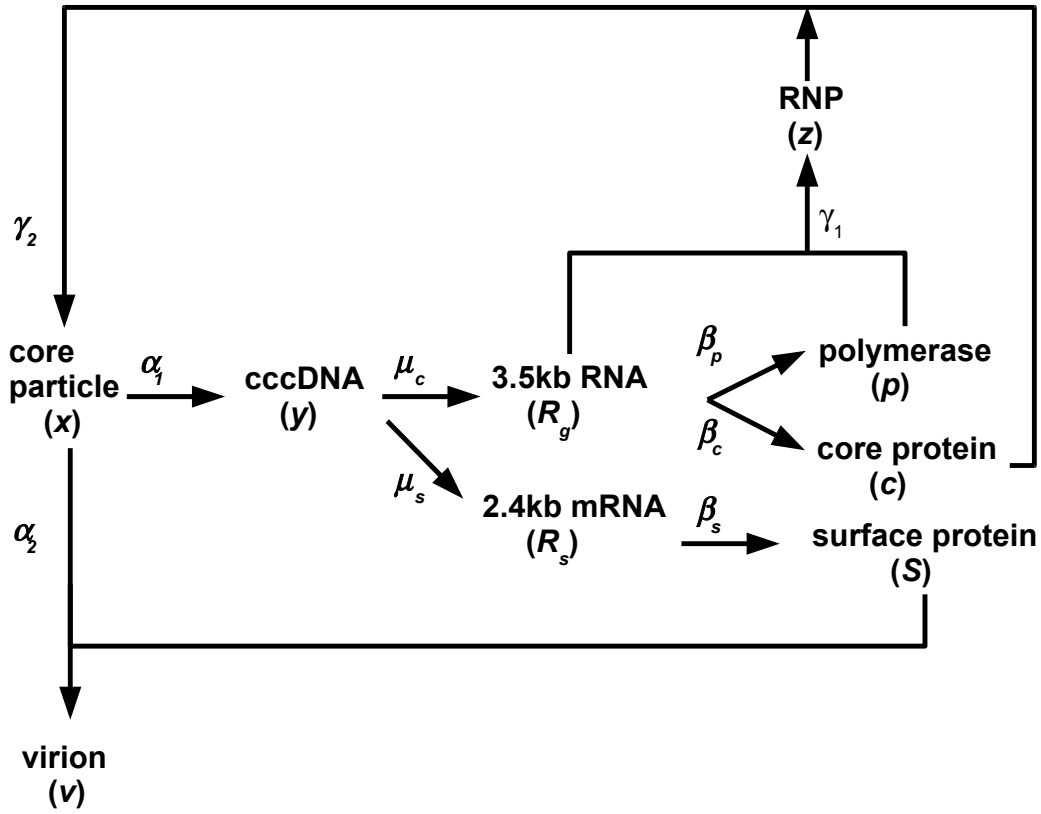

Figure 1: The intracellular replication process of HBV. The replication cycle has started when the core particle invades into the hepatocyte. Partially double stranded DNA in the core particle is repaired to complete closed circular DNA (cccDNA) by the viral polymerase packed in the core particle. And 3.5kb and 2.4kb mRNA are then expressed from cccDNA. Pre-genome RNA, viral polymerase mRNA and core protein mRNA are contained in 3.5kb RNA and contribute to produce the core particle. HBV genome DNA is replicated by the reverse transcription by using the pre-genome RNA as a template. The replicated pdsDNA is coated by the core protein with the viral polymerase to reproduce the core particle. On the other hand, 2.4kb mRNA is translated to the surface protein which is main component of the envelope. The core particle is encapsidated depending on the surface protein to produce the complete virion.

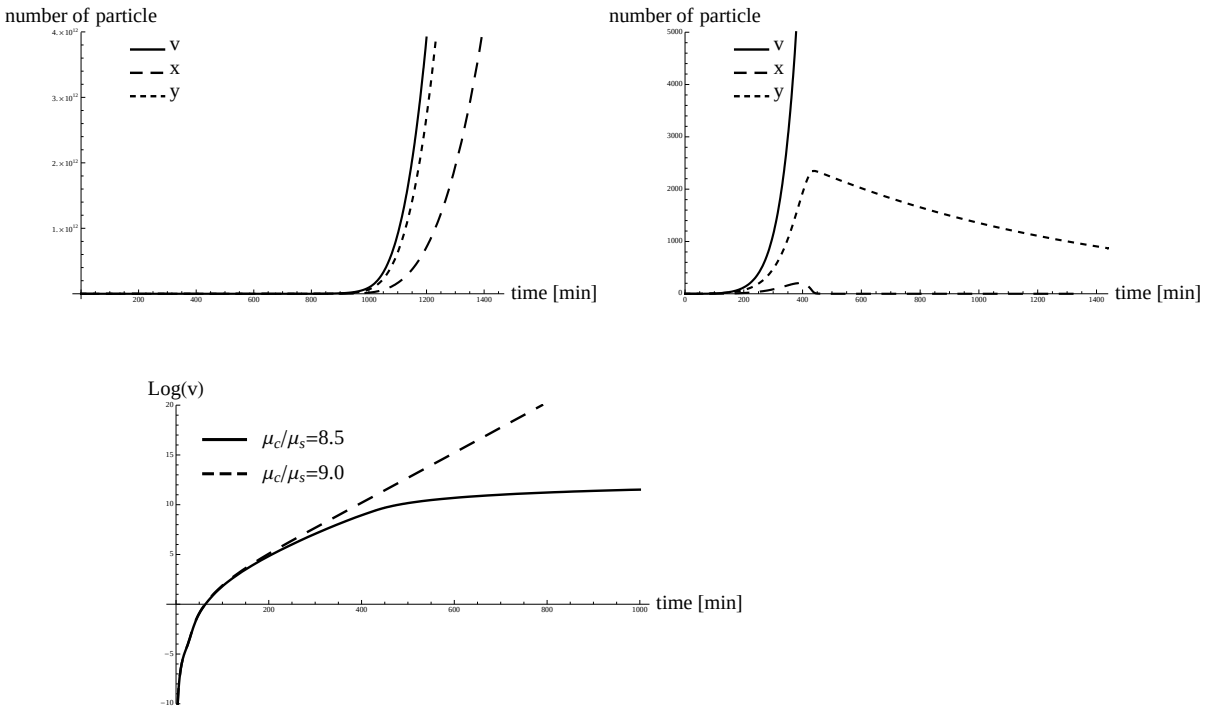

Figure 2: The time course of virion, core particle and cccDNA with various μ_c/μ_s. A: When μ_c/μ_s is sufficiently larger than a certain threshold, the core particle is accumulated and it can contribute to cccDNA replication. The number of virion is exponentially increased with the core particle and cccDNA by the positive feedback. B: The core particle converges to 0 when μ_c/μ_s is small. The cccDNA also converges to 0 because the supply of cccDNA from pdsDNA in the core particle stops. And the virion is then replicated until all cccDNA are degraded. The parameters are estimated by from the newly produced virion/day. Parameters:$\alpha_1 = 0.1[\text{min}^{-1}], \alpha_2 = 0.1[\text{molecules}^{-1}\text{min}^{-1}], \gamma_1 = 0.1[\text{molecules}^{-1}\text{min}^{-1}], \gamma_2 = 0.1[\text{molecules}^{-1}\text{min}^{-1}], \beta_p = \beta_c = \beta_s = 0.1[\text{min}^{-1}], \mu_c = 0.09[\text{min}^{-1}], \mu_s = 0.01[\text{min}^{-1}]$ for A. $\mu_c = 0.085[\text{min}^{-1}], \mu_s = 0.01[\text{min}^{-1}]$ for B. $\delta_x = \delta_y = \delta_z = \delta_{Rg} = \delta_{Rs} = \delta_c = \delta_p = \delta_s = \delta_v = 0.001[\text{min}^{-1}]$. C: Logarithmic plot of number of virion. The growth pattern is drastically changed depending on the ratio of μ_c to μ_s. HBV virion converge to a certain finite value when the ratio μ_c/μ_s is small. Finally, the replication cycle is arrested and the virion then decreases depending on the degradation rate δ_v. On the other hand, HBV can explosively grow when the ratio μ_c/μ_s exceeds a threshold level. Finally, HBV virion diverge to infinite.

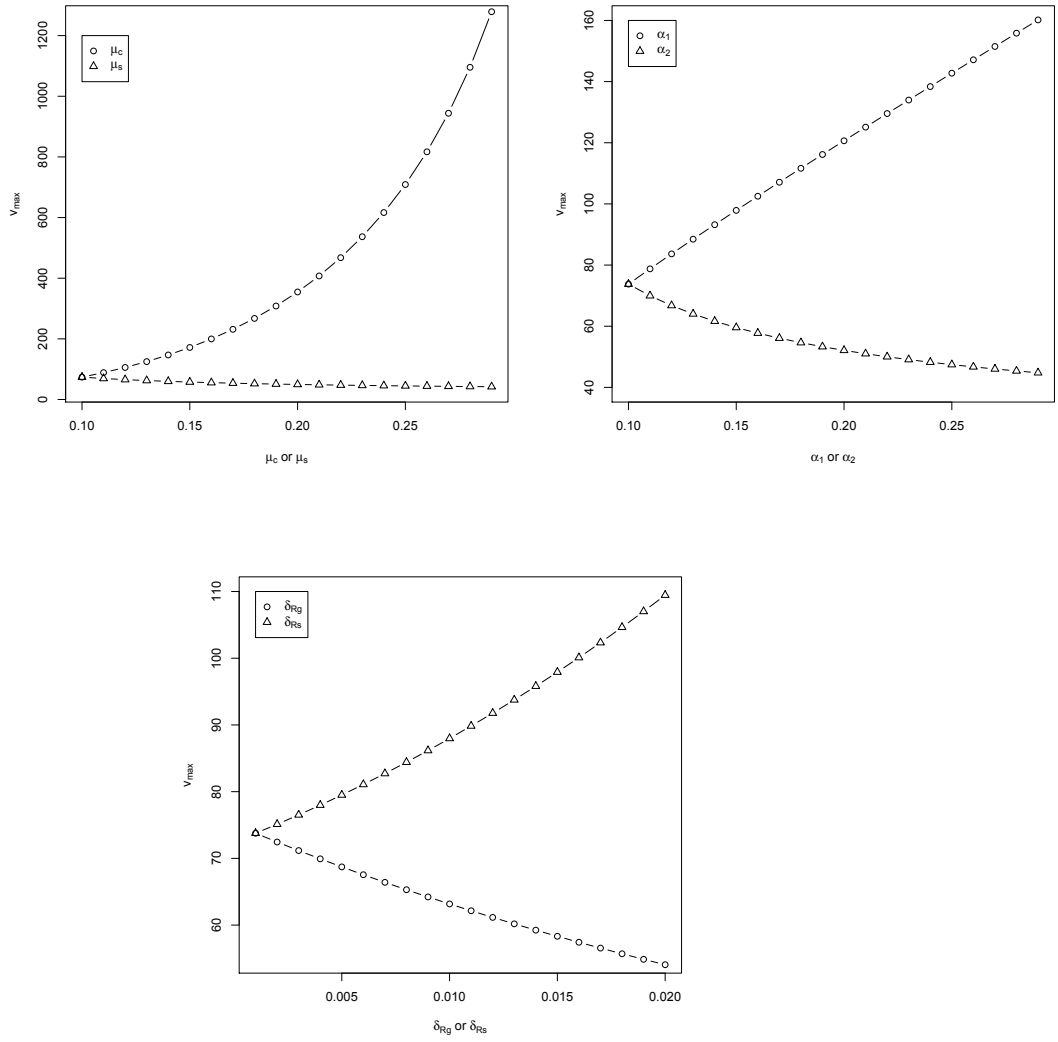

Figure 3: Parameter dependence of virion production. When parameters except for the selected one are fixed, maximum number of virion is obtained as the selected parameter is changed. The values of fixed parameters are the same in Fig 2A: The effect of the transcription rate for the virion production. Virion production increases or decreases as the transcription rate of 3.5kb RNA, μ_c, and 2.4kb mRNA, μ_s, increases, respectively. The effect of μ_c is significant as compared with other parameters. B: The effect of the reaction rate constant for the core particle or virion production. The virion production paradoxically decreases when the reaction rate constant for the interaction between the core particle and the surface protein to produce the virion, α_2, increases. C: The effect of the degradation rate of viral RNA. The virion production increases as the degradation rate of 2.4kb mRNA increases. The degradation of viral gene product can oppositely promote the virion production.

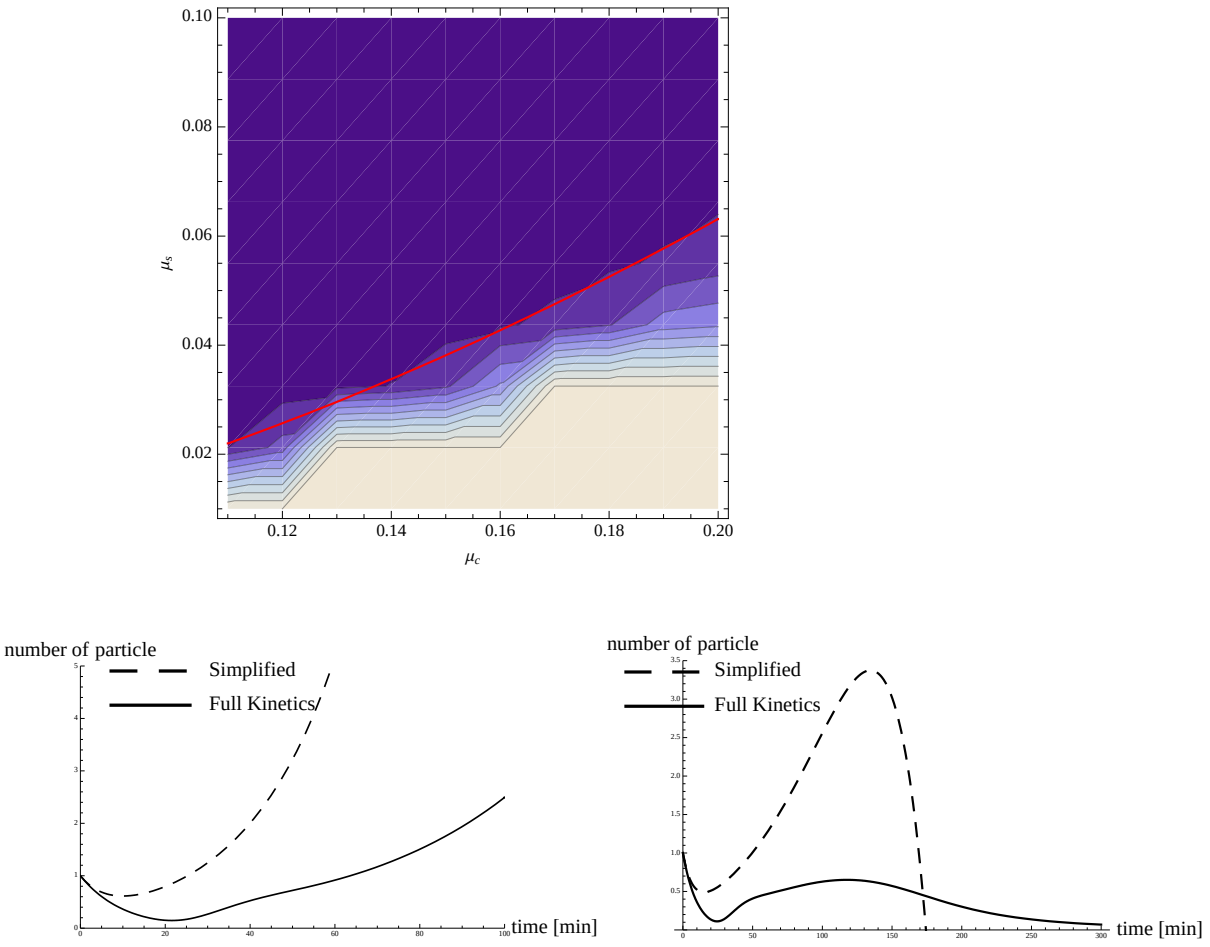

Figure 4: A: Threshold μ_c/μ_s ratio for explosive replication. Maximum concentration of virion are indicated as contour plot. The bright color indicates the region where the virion diverges to infinite. The threshold μ_c/μ_s ratio obtained from simplified model is plotted by red line. B: Time course of the core particle obtained from simplified model with large μ_c/μ_s ($\mu_c/\mu_s = 9$). The core particle diverges to infinite. The core particle obtained from simplified model proceeds to that from full kinetics model. This gap between these results is caused by ignoring the degradation term in simplified model. But it is conserved in both models that the core particle diverges to infinite after sufficiently long time has passed. C: Time course of the core particle obtained from simplified model with small μ_c/μ_s ($\mu_c/\mu_s = 6$). The core particle obtained from simplified model ahead becomes 0. But it is conserved in both models that the core particle becomes 0 after sufficiently long time has passed. The replication process is arrested when the core particle becomes 0 once.

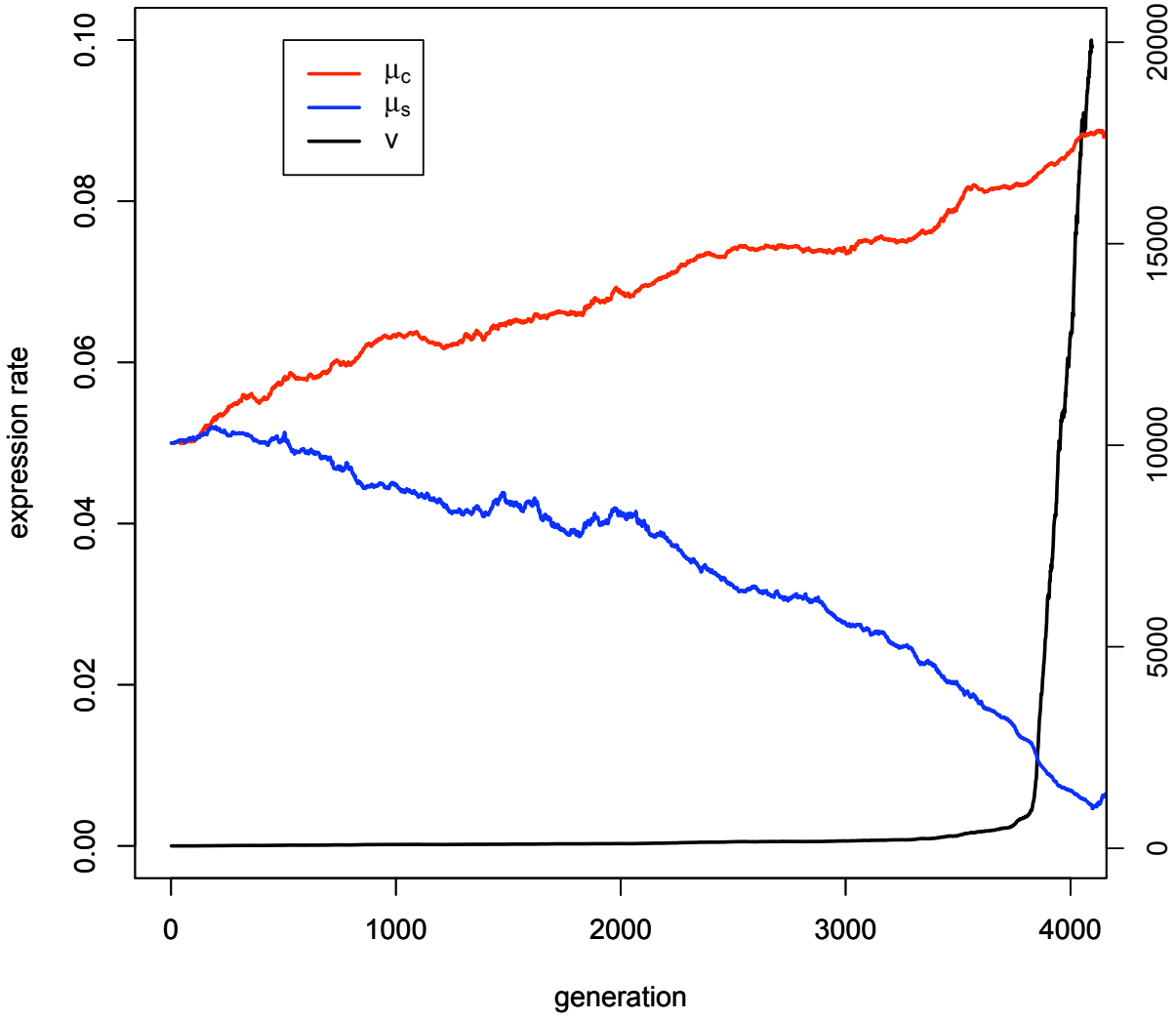

Figure 5: The evolutionary change of HBV within host. The frequency of HBV with high μ_c/μ_s increases as the generation proceeds. As mean of μ_c/μ_s increases, the number of virion increases. When μ_c/μ_s exceeds a threshold level, the virion prominently increases. Parameters: $J = 1000, \bar{\omega} = 0, \sigma = 0.001, N = 5000, \tau = 300$ [min].

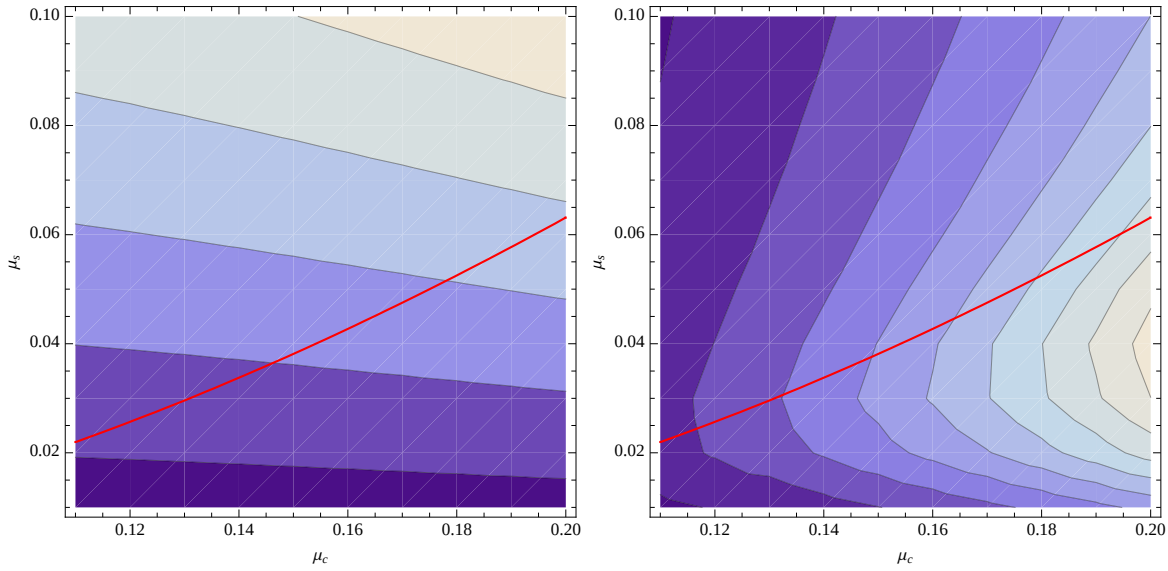

Figure 6: A: number of virion when waiting time τ for release of HBV virion from infected cell is small ($\tau = 20$ [min]). The number of virion is large when both μ_c and μ_s becomes high. It is expected that HBV evolves to right-top side where μ_c/μ_s becomes small. The optimum μ_c/μ_s ratio is smaller than the analytically obtained threshold for the explosive replication indicated by the red line. B: Number of virion when $\tau = 300$ [min]. The optimum μ_c/μ_s ratio maximizing $v(\tau)$ is larger than the threshold indicated by the red line. HBV can evolve to right-bottom side and HBV can explosively replicate.

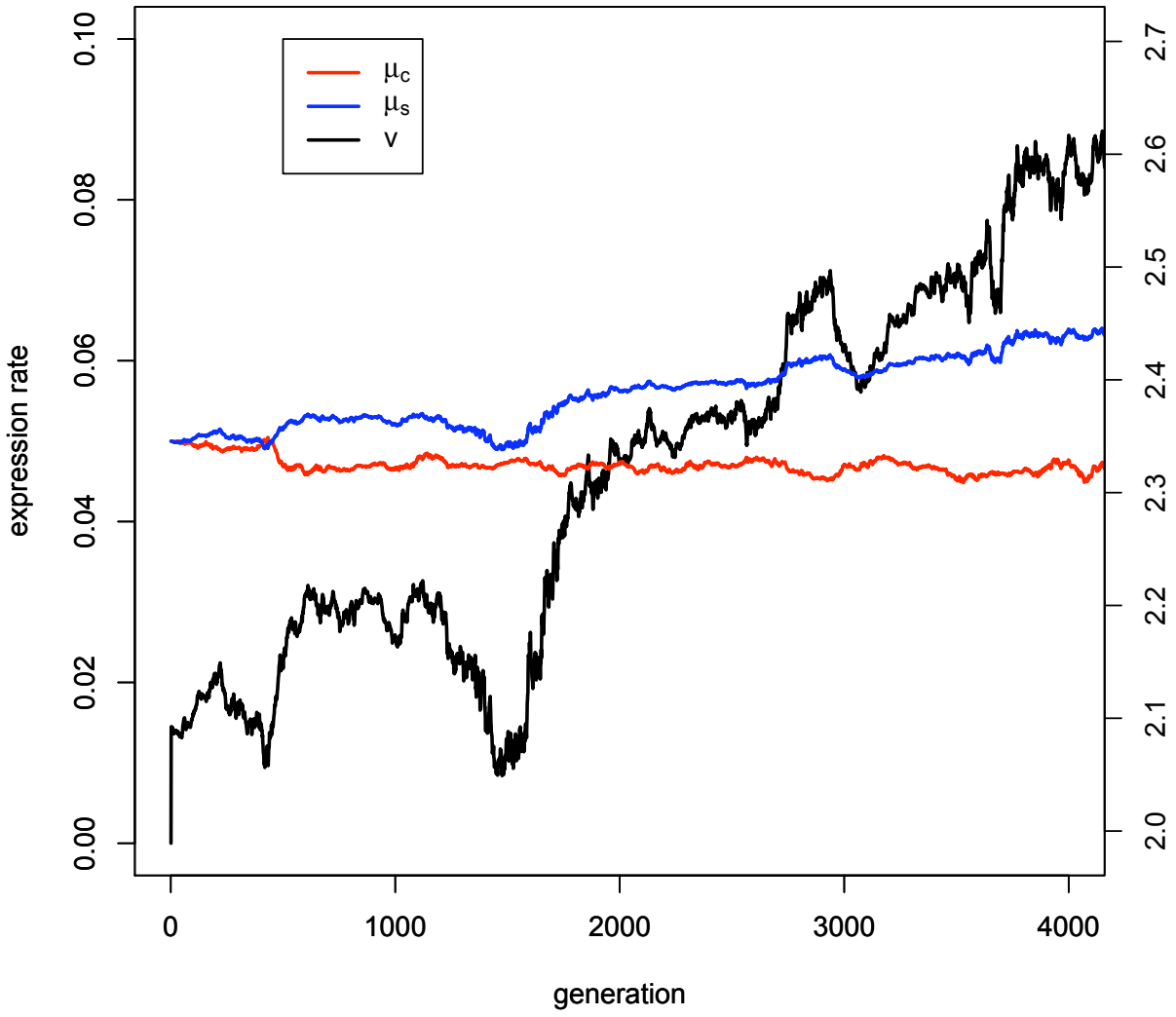

Figure 7: The evolutionary change of HBV within host when waiting time for HBV release is small. HBV with small μ_c/μ_s can evolve under this condition. The mean number of virion remain relatively small as compared with Fig 5. The chronic hepatitis is maintained without emergence of the explosive genotype of HBV. Parameters: $J = 1000, \bar{\omega} = 0, \sigma = 0.001, N = 5000, \tau = 20$[min].

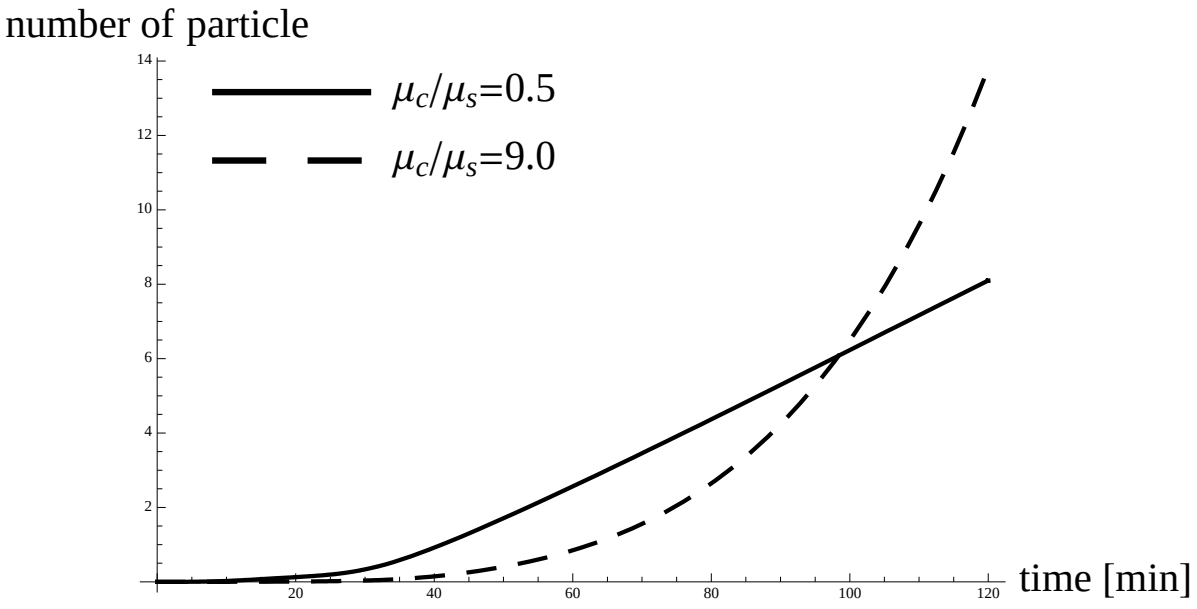

Figure 8: The trade-off between the initial speed and final production of the virion. The time course of the virion production of the arrested (solid line) or the explosive (dashed line) genotype. The number of the virion of the arrested type arises faster than that of the explosive type. But the number of virion of the explosive type exceeds to that of the arrested type after a while. When waiting time for HBV release is small, the arrested type of HBV becomes advantageous because the virion of explosive HBV genotype cannot be accumulated until the virion release from cell to expand the infection.

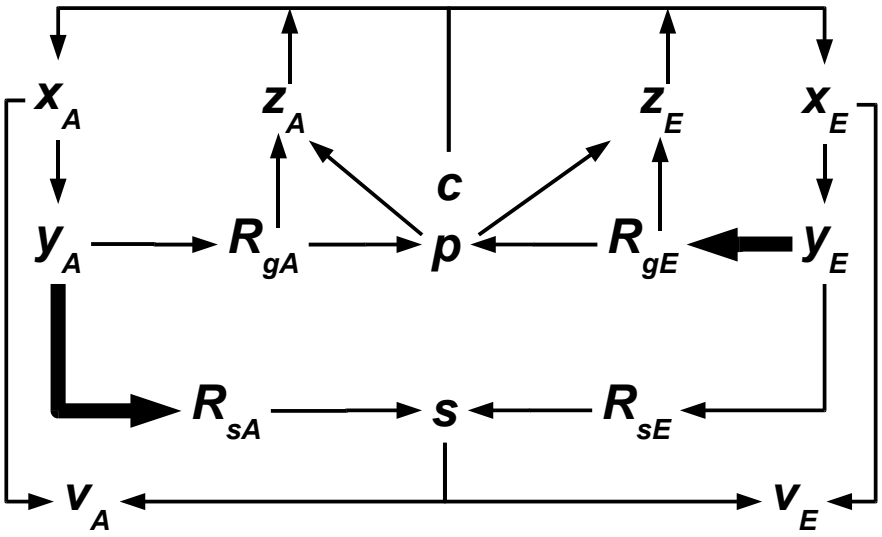

Figure 9: The diagram of HBV replication under super infection. One cell is infected by two genotypes of HBV, arrested and explosive genotype. The promoter activity of 2.4kb mRNA in arrested genotype is lager than that of 3.5kb RNA. Inversely, the promoter activity of 3.5kb RNA in explosive genotype is larger than that of 2.4kb mRNA. Core protein, polymerase and the surface protein are not distinguished because there is any difference in the coding sequence of the viral mRNA.

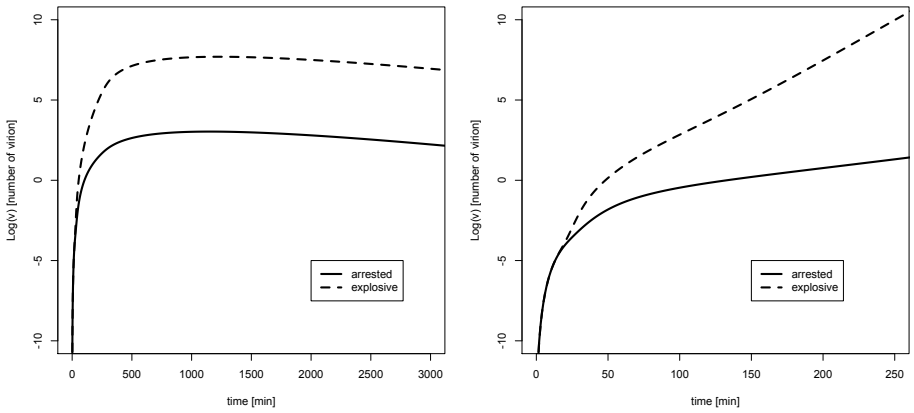

Figure 10: The effect of superinfection. A: Time course of the number of the virion when cell is simultaneously infected with both genotypes of HBV. The number of virion of explosive type (dashed line) cannot diverge to infinite in spite that ratio μ_{cE}/μ_{sE} satisfy the condition for the explosive replication ($\mu_{cE}/\mu_{sE} = 9$ and $\mu_{cA}/\mu_{sA} = 2$). The excessive production of the surface protein from arrested type (solid line) prevents the positive feedback of the explosive genotype. B: The number of virion of the explosive genotype (dashed line) can diverge to infinite under superinfection with the arrested genotype (solid line), when μ_c/μ_s extremely exceeds the threshold level ($\mu_{cE}/\mu_{sE} = 15$).

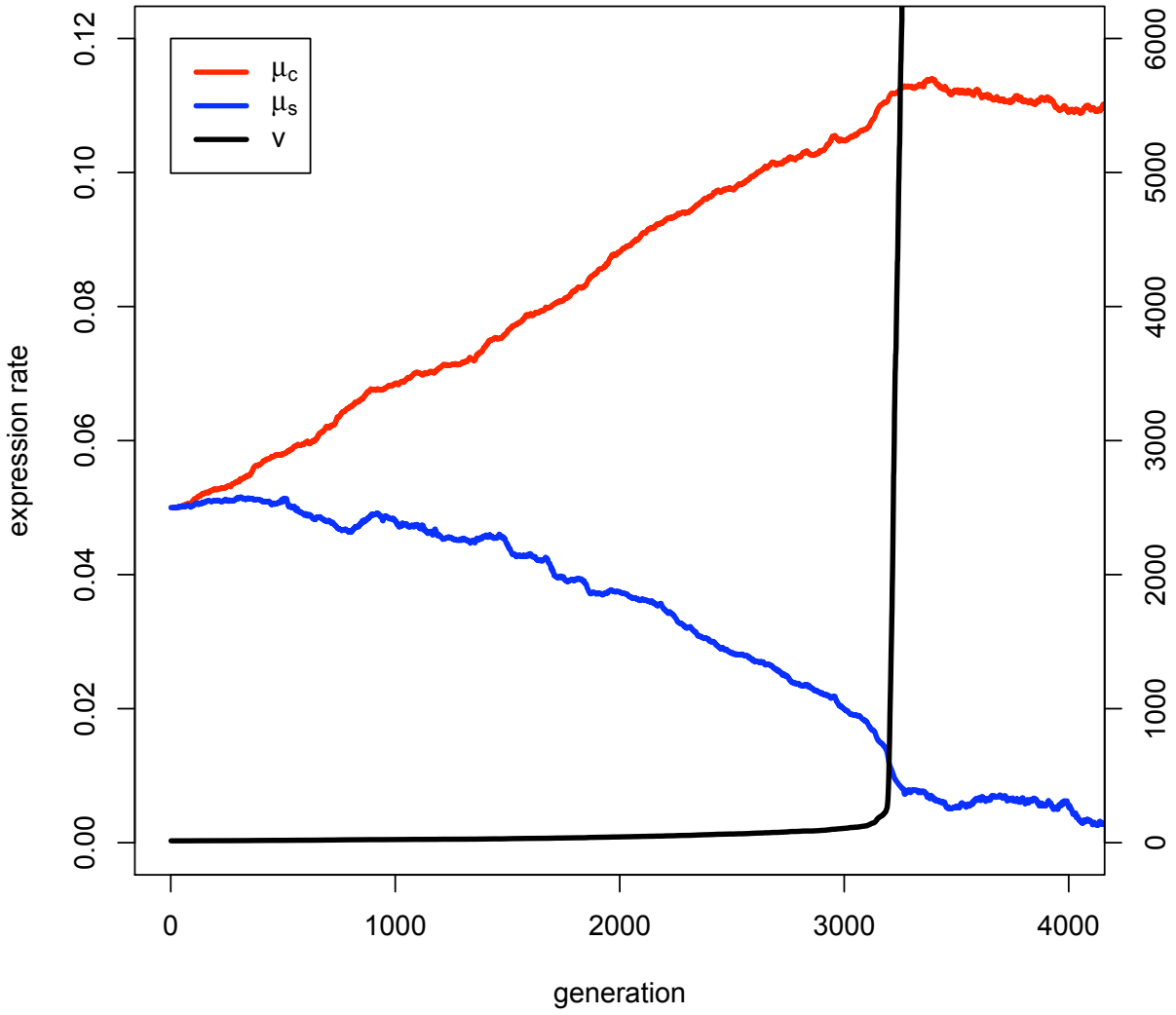

Figure 11: A sample path of the evolutionary simulation with superinfection. It is assumed that all hepatocytes are already infected by arrested type ($\mu_{cA}/\mu_{sA} = 0.5$). The mutant infects to hepatocyte already infected by arrested type. μ_{cA}/μ_{sA} ratio of arrested type is fixed. The mean value of μ_{cE} and μ_{sE} of mutants increases and decreases, respectively. For the explosive replication of HBV, μ_{cE}/μ_{sE} ratio of the mutant should become large exceeding the threshold level as well as shown in Fig 10B.