Plasmid Copy Number Control: A Case Study of the Quasi-Steady State Assumption

Lee A. Segel
Alan S. Perelson

SFI WORKING PAPER: 1991-08-045

SANTA FE INSTITUTE

PLASMID COPY NUMBER CONTROL: A CASE STUDY OF THE QUASI-STEADY STATE ASSUMPTION*

Lee A. Segel

Department of Applied Mathematics and Computer Science
Weizmann Institute
Rehovot, IL-76100 Israel

and

Alan S. Perelson

Theoretical Biology and Biophysics
Theoretical Division
Los Alamos National Laboratory
Los Alamos, NM 87545 U.S.A.

* Work performed under the auspices of the U. S. Department of Energy

PLASMID COPY NUMBER CONTROL: A CASE STUDY OF THE QUASI-STEADY STATE ASSUMPTION

Lee A. Segel and Alan S. Perelson

ABSTRACT

Perelson and Brendel (1989) have proposed kinetic models for the control of plasmid copy number, based on experiments by J. Tomizawa and his associates. The quasi-steady state assumptions (QSSA) made in the analysis of these models are justified in the present paper, thereby providing an example of how QSSA can provide a powerful and reliable tool in the analysis of biological kinetics.

1. Introduction

Virtually every biochemistry textbook shows how the "steady state assumption" can be employed in an analysis of a simple enzyme-substrate reaction to yield the familiar and extremely useful Michaelis-Menten approximation. Yet, only recently has it become clear precisely what conditions must be satisfied in order that this assumption be valid (Segel, 1988; Segel and Slemrod, 1989; see also Palsson, 1987.)

Of course the biochemist is usually faced with kinetics that are considerably more complicated than the reaction of an enzyme and a substrate that reversibly form a complex, which breaks down irreversibly to product and enzyme. Here we demonstrate that the above-cited analyses of the steady state assumption can with profit be extended to quite complex situations. For definiteness, we base our discussion on an important particular case, a theoretical analysis of extensive experiments concerned with sense anti-sense RNA interactions involved in plasmid ColE1 copy number control (Perelson and Brendel, 1989 – referred to from now on as PB). Our analysis reinforces PB's numerical simulations and establishes the parameter regime in which the steady state assumption is valid.

Theoreticians prefer the term quasi-steady-state assumption (QSSA). This term emphasizes the fact that no concentrations are imagined to be in a true steady state (where, by definition, they would be constant). Rather, certain concentrations are assumed to change in a special manner so as to be in a "steady state" with the changing values of certain other (slowly varying) concentrations. The steady state ensues after a

transient whose duration t_f is brief compared to the time scale t_s for significant change in the slowly varying concentrations. A major step in a careful examination of the QSSA is to estimate t_f and t_s in terms of the parameters of the problem and thereby to ensure that $t_f \ll t_s$ for the parameter range of interest. One should also verify that the allegedly slowly varying quantities in fact undergo negligible change during the fast transient. We carry out these steps for the copy number control example. Our exposition is reasonably self contained, but for fuller understanding the reader should consult the papers by Segel (1988) and Segel and Slemrod (1989).

2. Plasmid Copy Number Control

Elucidation of the mechanisms underlying control of plasmid copy number is of interest for a number of reasons (c.f. Scott, 1984). Under suitable experimental conditions the presence of plasmids is not essential for the viability of their bacterial host. Plasmid replication therefore, unlike chromosomal replication, can be experimentally perturbed without being lethal to the cell and thus serves as a model system for studying the regulation of DNA replication. Plasmids confer to their hosts properties such as resistance to antibiotics or altered metabolic capacity that are of medical, industrial, and agronomical importance. Furthermore, plasmids have become widely employed vectors for the cloning of genes desired in large quantities for use in research applications and in pharmaceutical production, so that increasing plasmid copy number is an important research goal (Ausubel et al., 1987).

The number of plasmids per cell is called the plasmid copy number. As the host bacteria divides, the number of plasmids per cell decreases. Plasmids with high copy numbers, such as ColE1, seem to segregate at random between the two daughter cells, so that on average each daughter gets half the plasmids. Copy number control thus involves ensuring that a plasmid normally maintained at an average of N copies per cell replicates an average of N times per cell generation. Because plasmid replication takes only a small portion of the cell cycle, control of plasmid copy number occurs at the level of initiation of plasmid DNA replication.

The molecules involved in the replication control of the ColE1 family of plasmids have been extensively studied. We briefly summarize the known molecular details (for a review see Cesareni & Banner, 1985; a model incorporating most of these findings is presented by Brendel & Perelson, 1992). In order for plasmid DNA to replicate, a piece of RNA needs to hybridize with the DNA near the origin of replication. This RNA is called a primer, and is required for the initiation of DNA synthesis by DNA polymerase I (Itoh & Tomizawa, 1980). The primer is generated by an RNA polymerase that transcribes an RNA molecule, called RNA II, from an initiation site 555 base pairs upstream of the origin of replication (Fig. 1). More than half of the nascent RNA II transcripts that extend beyond the origin of replication form a persistent hybrid with the template DNA near the origin (Tomizawa & Itoh, 1981; Masukata & Tomizawa,

1986). Cleavage of a hybridized RNA II transcript at the origin by the enzyme RNAase H yields a primer for the initiation of DNA synthesis (Itoh & Tomizawa, 1980; Selzer & Tomizawa, 1982; Masukata & Tomizawa, 1984). Once a primer is formed, DNA replication rapidly follows. Thus replication control involves inhibiting the formation of the primer. Inhibition occurs by the interaction of a second, shorter, RNA molecule, called RNA I, transcribed in the direction opposite to that of RNA II beginning 445 base pairs upstream from the origin of DNA replication (Fig. 1). Because RNA I and RNA II are transcribed from opposite strands of the DNA, they are complementary and can interact to form an RNA I – RNA II hybrid (Tomizawa, 1984; Tamm & Polisky, 1985). The binding of RNA I to RNA II prevents the RNA II from forming a stable hybrid with the template DNA at the origin of replication. Consequently primer formation and plasmid replication are inhibited (Masukata & Tomizawa, 1986; Tomizawa, 1986).

A 63 amino acid protein, called Rom or Rop, encoded downstream from the origin of replication, increases the interaction of RNA I with RNA II and thereby increases inhibition of replication (Cesareni, et al., 1982; Som & Tomizawa, 1983; Tomizawa & Som, 1984; Tomizawa, 1985, 1986). The importance of Rom can be shown by deletion of the Rom gene, which results in an increase in plasmid copy number (Twigg & Sherratt, 1980).

The interactions among RNA, RNA II and Rom protein have been studied in great detail *in vitro* (Tomizawa, 1984, 1985; Tomizawa & Som, 1984; Dooley & Polisky, 1987). A kinetic model of these interactions has been developed by Perelson and Brendel (1989). It is this model that we analyze below, with emphasis on justifying the ad hoc QSSAs made in that paper.

3. The "Kissing" Model

RNA I and RNA II are thought to fold into the typical stem loop structures characteristic of RNAs. Thus even though RNA I and RNA II contain complementary base sequences, many of the bases may already be paired within a single molecule. Tomizawa (1984) has suggested that the first interaction between RNA I and II is between single stranded regions found on the loops of the molecules. This loop – loop interaction has been termed "kissing", and is thought to generate a rather weakly bounded state. Following this initial interaction conformational changes may occur that allow further base pairing and the formation of a stable RNA I – RNA II complex.

A kinetic scheme proposed by PB that encompasses this picture of RNA I – RNA II interaction is

$$R_I + R_{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \quad , \tag{3.1}$$

where C^* is a short-lived intermediate, i.e., the kissing state, and C is a stable complex. Because C can reform C^*, it is probably not a fully based paired duplex. We will return to this point later.

PB analyzed the experiments of Tomizawa and Som (1984) in which the kinetics of RNA I – RNA II interaction *in vitro* were determined. In these experiments, as well as *in vivo*, the concentration of R_I is sufficiently large compared with the concentration of R_{II} that it varies only negligibly from its initial amount (Brenner & Tomizawa, 1991). Thus we assume

$$R_I \approx R_I^0 \quad . \tag{3.2a}$$

If R_{II}^0 is the initial amount of R_{II}, and if C and C^* are initially absent, then mass conservation gives

$$R_{II} = R_{II}^0 - C^* - C \quad . \tag{3.2b}$$

Employing (3.2b) and the law of mass-action, we can easily derive the equations governing the kinetics of the reactions in (3.1):

$$\frac{dC^*}{dt} = \alpha - \beta C^* - \gamma C \quad , \quad \frac{dC}{dt} = k_2 C^* - k_{-2} C \quad , \tag{3.3a,b}$$

with initial conditions

$$C(0) = C^*(0) = 0 \quad , \tag{3.3c,d}$$

where

$$\alpha \equiv k_1 R_I^0 R_{II}^0 \quad , \quad \beta \equiv k_1 R_I^0 + k_{-1} + k_2 \quad , \quad \gamma \equiv k_1 R_I^0 - k_{-2} \; . \tag{3.3e,f,g}$$

We expect that the concentration of the short-lived intermediate C^* will change rapidly during an initial transient period, after which C^* will be in a quasi-steady state

with the stable complex C. To obtain the QSSA, we follow the standard procedure of setting the right side of (3.3a) to zero, obtaining

$$C^* = \frac{\alpha - \gamma C}{\beta} \quad . \tag{3.4}$$

Substituting (3.4) into (3.3b), we find

$$\frac{dC}{dt} = k_2 \left[\frac{\alpha - \gamma C}{\beta} \right] - k_{-2} C \quad .$$

This gives the QSSA equation

$$\frac{dC}{dt} = \frac{k_2 \alpha}{\beta} - \delta C \quad , \tag{3.5a}$$

with solution

$$C(t) = \frac{k_2 \alpha}{\beta \delta} \left(1 - e^{-\delta t}\right) \quad , \tag{3.5b}$$

where we have used the standard initial condition $C(0) = 0$, and

$$\delta \equiv k_{-2} + (k_2 \gamma / \beta) \quad . \tag{3.5c}$$

According to (3.5b), C changes exponentially with halftime t_s, where

$$t_s \approx \delta^{-1} \quad , \tag{3.6a}$$

toward a steady state

$$C_{ss} = \frac{k_2 \alpha}{\beta \delta} \quad . \tag{3.6b}$$

We now analyze the conditions under which the QSSA is a good assumption. First, we require that the intermediate C^* change rapidly during an initial transient, reaching a quasi-steady state with the stable complex C. To obtain the behavior during the initial fast transient, we put $C = 0$ in (3.3a):

$$\frac{dC^*}{dt} = \alpha - \beta C^* \quad . \tag{3.7a}$$

According to (3.7a), in a fast transient of duration t_f, where

$$t_f \approx \beta^{-1} \quad , \tag{3.7b}$$

C^* reaches a steady state

$$\overline{C}^* = \frac{\alpha}{\beta} \quad . \tag{3.7c}$$

This steady state is consistent with the one predicted by the QSSA (3.4) at early times, when $C = 0$.

One necessary condition for the QSSA is that the fast time scale t_f in fact be fast when compared with the slow scale t_s, i.e.,

$$t_f \ll t_s \ , \quad \text{or equivalently,} \quad \beta \gg \delta \quad . \tag{3.7d}$$

We have used the standard initial condition for the QSSA equation (3.5), $C(0) = 0$. For this to be a good approximation, we must have

$$C_f / C_{ss} \ll 1 \quad , \tag{3.8}$$

where C_f is an estimate of the value of C at the end of the fast transient. From (3.3b) with $C = 0$, we obtain the following estimate of dC/dt during the fast transient:

$$dC/dt \approx k_2 C^* \quad . \tag{3.9}$$

Note that the approximation (3.9) is conservative, since dC/dt is overestimated. From (3.9), then, an approximate estimate for C at the termination of the fast transient is

$$C_f \approx k_2 \overline{C}^* t_f \quad . \tag{3.10a}$$

Thus, from (3.7c) and (3.6)

$$\frac{C_f}{C_{ss}} \approx \frac{t_f}{t_s} \quad . \tag{3.10b}$$

Consequently, condition (3.8) is guaranteed by (3.7d).

In terms of the original parameters of the problem, condition (3.7d) is

$$k_1(K_m + R_I^0)^2 \gg k_2 R_I^0 + k_{-2}(K_m + R_I^0) - (k_2 k_{-2}/k_1) \quad , \tag{3.11a}$$

where

$$K_m \equiv (k_{-1} + k_2)/k_1 \quad . \tag{3.11b}$$

In the present problem, according to the parameter estimates of PB (see Table 1)

$$R_I^0 \ll K_m \quad , \tag{3.12}$$

in which case, upon approximating $K_m + R_I^0$ by K_m, dividing by $k_2 K_m$, again applying (3.12) and using the definition of K_m, we find that (3.11) reduces to

$$1 + \frac{k_{-1}}{k_2} + \frac{k_{-2}}{k_{-1} + k_2} \gg \frac{k_{-2}}{k_2} \quad . \tag{3.13}$$

A sufficient condition for (3.13) is

$$k_{-2}/k_2 \ll 1 \quad , \tag{3.14}$$

which is the case according to the PB parameter estimates (Table 1).

For completeness, we verify assumption (3.2a) that $R_I \approx R_I^0$ under our assumption

$$R_{II}^0 \ll R_I^0. \tag{3.15}$$

From (3.2b) and (3.15) it follows that

$$C^* < R_{II}^0, \; C < R_{II}^0 \quad \text{and hence } C^* \ll R_I^0, \; C \ll R_I^0. \tag{3.16a-d}$$

That $R_I \approx R_I^0$ now follows immediately from the conservation law

$$R_I + C^* + C = R_I^0 \quad , \tag{3.17}$$

which is implied by the kinetic scheme (3.1).

4. Enhancement by the Rom Protein

Although the interaction between RNA I and RNA II seems sufficient to control plasmid copy number, the wild type ColE1 plasmid contains a gene for the Rom protein. In the presence of this protein, RNA I seems to be a more effective inhibitor of plasmid replication and the plasmid copy number is reduced when compared with mutants that lack an intact Rom gene. To understand how Rom may act, PB suggested that Rom interacts with the unstable complex C^* and enhances its transition to the stable complex C. Using the kinetic scheme

$$R_I + R_{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \quad , \quad C^* + M \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_M \overset{k_4}{\longrightarrow} C + M \quad ,$$

and making QSSA's on both C^* and C_M, PB were able to fit kinetic data from Tomizawa and Som (1984) on the action of Rom in both wildtype and mutant plasmids. Here we analyze the conditions under which this double QSSA is justified.

It is convenient here to introduce the dimensionless variables

$$\tau \equiv k_1 M t \quad , \quad c^* \equiv C^*/M \quad , \quad c_M = C_M/M \quad , \quad c = C/M \quad , \quad r_{II} = R_{II}/M \quad . \tag{4.1}$$

In choosing the dimensionless variables in (4.1), we have used the fact that in the experiments Rom concentration is in excess, so that M can be regarded as a constant. Also, R_I is regarded as constant, as in (3.2).

In terms of the variables of (4.1), the above kinetic scheme is described by the equations

$$\begin{aligned} dc^*/d\tau &= r_I^0 r_{II} - \mu c^* + \kappa_{-2} c - \zeta c^* + \kappa_{-3} c_M \quad , \\ dc_M/d\tau &= \zeta c^* - \nu c_M \quad , \\ dc/d\tau &= \kappa_2 c^* - \kappa_{-2} c + \kappa_4 c_M \quad , \\ r_{II} &= r_{II}^0 - c^* - c_M - c \quad , \end{aligned} \tag{4.2a-d}$$

with initial conditions

$$c^*(0) = c_M(0) = c(0) = 0 \quad , \quad r_{II}(0) = r_{II}^0 \quad . \tag{4.2e-h}$$

In (4.2) we employed the following dimensionless parameters (not all of which are independent):

$$r_I^0 = R_I^0/M \quad , \quad r_{II}^0 = R_{II}^0/M \quad ; \tag{4.3a,b}$$

$$\frac{k_i}{k_1 M} \equiv \kappa_i \quad , \quad i = -1, 2, -2, -3, 4 \quad ; \tag{4.3c}$$

$$\frac{k_3}{k_1} \equiv \zeta \quad , \quad \frac{K_m}{M} \equiv \mu \quad , \quad \frac{\hat{K}_m}{M} \equiv \nu \quad , \tag{4.3d-f}$$

where K_m is defined in (3.11b) and

$$\hat{K}_m = (k_{-3} + k_4)/k_1. \tag{4.3g,h}$$

Now we have two relatively fast intermediates, c^* and c_M, which are expected to rapidly approach a quasi-steady state. Paralleling our derivation of (3.4a), to find the fast time scale we set $c = 0$ in (4.2a) and (4.2b). If the resultant linear equations have a solution proportional to exp (λt), then λ can be found from the determinental equation

$$\begin{vmatrix} -(r_I^0 + \mu + \zeta) - \lambda & \kappa_{-3} - r_I^0 \\ \zeta & -\nu - \lambda \end{vmatrix} = 0 \quad . \tag{4.4}$$

The two roots of (4.4) will be somewhat complicated combinations of the various parameters. We will simplify the roots under the assumption that M is large. This will produce formulas that are valid in the most important parameter range.

Note that only the smaller root, λ, interests us, for this corresponds to the rate limiting relatively slow processes in the fast transient region. In seeking a simple criterion, we shall also simplify the formulas under the assumption that R_I^0 is suitably small, for again this will turn out to be the case of maximal interest. [If necessary, however, the counterparts of the key formulas (4.11) and (4.16) below can be obtained straightforwardly without assumptions as to the relative magnitudes of the various parameters.]

In the quadratic (4.4), all parameters except ζ approach zero as $M \to \infty$. Thus, for large M the quadratic can be approximated by

$$\lambda^2 + \zeta\lambda + \zeta(\nu - \kappa_{-3} + r_I^0) = 0 \quad . \tag{4.5}$$

We observe that

$$\nu - \kappa_{-3} = \kappa_4 \quad . \tag{4.6}$$

We further assume that

$$r_I^0 \ll \kappa_4 \quad ; \quad \text{i.e., } R_I^0 \ll k_4/k_1 \quad . \tag{4.7}$$

The roots of the quadratic are thus approximated by

$$2\lambda = -\zeta \pm \left[\zeta^2 - 4\kappa_4\zeta\right]^{\frac{1}{2}} \quad . \tag{4.8}$$

The desired smaller root satisfies

$$\lambda \approx -\kappa_4 \quad . \tag{4.9}$$

Thus the dimensionless fast time scale is

$$\tau_{\text{fast}} = 1/\kappa_4 \quad . \tag{4.10a}$$

Since time has been made dimensionless with $1/k_1 M$ [see (4.1)], the dimensional fast time scale is given by

$$t_f = 1/(k_1 M \kappa_4) = 1/k_4 \quad . \tag{4.10b}$$

At the end of the fast transient, we find from (4.2a,b) with $d/dt = 0$ and $c = 0$ that the intermediates have the following approximate values:

$$\bar{c}^* = \frac{r_I^0 r_{II}^0 k_f}{\kappa_2 + \kappa_4\zeta\nu^{-1}} \quad , \quad \bar{c}_M = \zeta\nu^{-1}\bar{c}^* \quad ; \tag{4.11a,b}$$

Here

$$k_f = \frac{\kappa_2 + \kappa_4 \zeta/\nu}{\mu + r_I^0 + (\kappa_4 + r_I^0)\zeta/\nu} \quad . \tag{4.11c}$$

We now turn to the period following the fast transient. After making quasi-steady state assumptions on c_M and c^*, we obtain for c the simple equation

$$dc/d\tau = k_f r_I^0 r_{II}^0 - \left(k_r + k_f r_I^0\right) c \quad , \tag{4.12a}$$

where

$$k_r = \frac{\kappa_{-2}\left[\kappa_{-1} + r_I^0(1 + \zeta\nu^{-1})\right]}{r_I^0 + \mu + (r_I^0 + \kappa_4)(\zeta/\nu)} \quad . \tag{4.12b}$$

For large M

$$k_f \approx \frac{\kappa_4 \zeta/\nu}{(\kappa_4 + r_I^0)(\zeta/\nu)} \approx 1 \quad , \quad k_r \approx \frac{\kappa_{-2} r_I^0}{\kappa_4} \quad , \tag{4.13a,b}$$

where we have again employed (4.7).

From (4.12a), we see that the stable complex c exponentially approaches the steady state

$$c_{ss} = \frac{k_f r_I^0 r_{II}^0}{k_r + k_f r_I^0} \tag{4.13c}$$

with a dimensionless e-folding time of $(k_r + k_f r_I^0)^{-1}$. With the simplifications (4.13a,b), this yields a dimensional slow time scale of

$$t_s^{-1} = k_1 R_I^0 \left[1 + \frac{k_{-2}}{k_4}\right] \quad . \tag{4.14}$$

The necessary condition

$$t_f \ll t_s \tag{4.15}$$

is, using (4.10b),

$$R_I^0 \ll \frac{k_4}{k_1} \Big/ \left[1 + \frac{k_{-2}}{k_4}\right] \quad . \tag{4.16}$$

Moreover, by suitable generalization of the calculations in Section 2, one can again establish (3.10b), so that (4.15) also assures that the standard initial condition $c(0) = 0$ is suitable for (4.12a).

Table 1. Parameter values from PB

parameter	wild type plasmid	inc1 inc2 mutant
k_1	$9 \times 10^7 M^{-1}\,\text{min}^{-1}$	$7.4 \times 10^7 M^{-1}\,\text{min}^{-1}$
k_{-1}	$28.4\,\text{min}^{-1}$	$37.6\,\text{min}^{-1}$
k_2	$25.8\,\text{min}^{-1}$	$6.6\,\text{min}^{-1}$
k_{-2}	$0.05\,\text{min}^{-1}$	$0.1\,\text{min}^{-1}$
k_3	$10^8 M^{-1}\,\text{min}^{-1}$	$1.95 \times 10^7 M^{-1}\,\text{min}^{-1}$
k_{-3}	$0.1\,\text{min}^{-1}$	$0.1\,\text{min}^{-1}$
k_4	$20\,\text{min}^{-1}$	$20\,\text{min}^{-1}$
R_I^0	$6 \times 10^{-9} M$	$6 \times 10^{-9} M$
R_{II}^0	$10^{-10} M$	$10^{-10} M$
M	$4 \times 10^{-6} M$	$4 \times 10^{-6} M$

From Table 1 we see that for both wild type and the *inc*1*inc*2 mutant plasmid (4.16) can be well approximated by

$$R_I^0 \ll k_4/k_1 \quad , \tag{4.17}$$

which is the final specification of the parameter domain wherein the QSSA is permitted. Table 1 also shows that condition (4.17) is indeed satisfied.

There remains to verify the conditions that allowed us to derive the simplified form (4.9) of the smaller root of the quadratic (4.4). Condition (4.7) is the same

as (4.19), while "large M" turns out to require that $(k_3/k_1)M$ be large compared to K_m, R_I^0, and $\hat{K}_m$. From Table 1 we find that the largest of these last three quantities is $\hat{K}_m \approx 6 \times 10^{-7} M$. Since the experiments were carried out with $(k_3/k_1)M$ in the range $10^{-6}M$ to $3 \times 10^{-5}M$, the large M approximation is indeed appropriate.

Verification of the assumption $R_I \approx R_I^0$ when $R_{II}^0 \ll R_I^0$ proceeds analogously with the discussion of this point in Section 2. For the present model, however, the additional question arises of justifying the assumption that the Rom concentration M can be regarded as constant in the analysis. From the conservation law $M + C_M =$ constant, the required condition is that, at the end of the fast transient when C_M is maximal,

$$C_M \ll M, \quad \text{i.e.,} \quad c_M \ll 1 \ . \tag{4.18}$$

Employing (4.11) and (4.3) we find that (4.18) is equivalent to

$$R_I^0 R_{II}^0 - \frac{k_{-3}+k_4}{k_3}(K_m + R_I^0) \ll \frac{M k_3}{k_{-3}+k_4}[\frac{k_{-3}+k_4}{k_3}\left(\frac{k_4}{k_1} + R_I^0\right) - R_I^0 R_{II}^0 \frac{k_4}{k_2}] \ . \tag{4.19}$$

We simplify the above equation by employing the relations

$$R_I^0 \ll \frac{k_4}{k_1} \ , \quad R_I^0 \ll K_m \ , \quad k_{-3} \ll k_4 \ , \quad R_{II}^0 \ll \frac{k_4}{k_3} \tag{4.20}$$

which hold for the PB parameters. This yields

$$R_I^0 R_{II}^0 \ll \frac{k_2 k_4}{k_1 k_3} + \frac{k_2 k_4}{k_3^2}\frac{K_m}{M} \tag{4.21}$$

as the condition that justifies the assumption that "M is large enough so that it can be regarded as constant." Because of (4.17) and

$$R_{II}^0 \ll k_2/k_3 \ , \tag{4.22}$$

which also holds for the PB parameters, for the present case (4.21) holds for *all* values of the Rom concentration M.

5. Discussion

The QSSA is perhaps the most powerful single tool available to the theoretical kineticist. When applicable, use of the QSSA often provides formulas that are relatively simple in form and transparent to interpret, and hence are suitable for confrontation with experiment. In fact, BP used the QSSA to great benefit to extract parameter estimates from experimental data. Careful study of the QSSA on several examples should therefore be of interest.

Here we have illustrated how the QSSA can be justified by estimating the slow time scale t_s, the fast time scale t_f, and the concentrations of intermediates at the end of the fast transient. Moreover, we have demonstrated that these estimates are useful for purposes in addition to those for which they were originally derived. For example, employing our estimates of intermediate concentrations, we checked the consistency of other (genuine steady-state) approximations used in the case study – assuming that certain reactants were in excess and hence could be regarded as constants. As a further example of the use of the estimates of the slow time scale, let us investigate what these estimates tell us about the fact that the addition of Rom accelerates the formation of the complex C. From (3.6a) and (4.14), we note that the two slow time scales, without and with Rom, are given by

$$t_s^{-1} = k_1 R_I^0 / \left[1 + (k_{-1}/k_2)\right] \text{ and } t_s^{-1} = k_1 R_I^0 \ , \qquad (5.1a,b)$$

respectively. In obtaining (5.1b), we used the finding that the $C \rightarrow C^*$ back reaction is weak (k_{-2} relatively small). We can deduce from (5.1b) that in the presence of Rom, the intermediate C^* very rapidly transforms into C, so only the formation of C^* is rate limiting. In fact, the maximum rate of formation of C^* is $k_1 R_I^0$, which sets the slow time scale. We also see from (5.1a) that without Rom, transformation of C^* into C is strongly affected by the $C^* \rightarrow R_I + R_{II}$ back reaction (via k_{-1}), as well as by the time it takes for C^* to become C (via k_2).

A further approximation was made in the case study analysis, one that has not been discussed so far. No consideration has been taken of the step

$$C \xrightarrow{k} D \qquad (5.2)$$

by which the complex C changes irreversibly, albeit slowly, into a duplex molecule D in which RNA I is completely base-paired to RNA II. But if the time scale of this

irreversible step is long compared to the estimates (5.1) for t_s, then the final stage of the reaction can be described by

$$\frac{dD}{dt} = kC \ , \quad D(0) = 0 \ . \tag{5.3a, b}$$

Because the $C \longrightarrow D$ step is slow, we can replace C in (5.3) by a steady state value. For example, if the scheme (3.1) is supplemented by the slow step (5.2), then employment of the conservation law

$$R_{II}^0 = R_{II} + C^* + C + D \ , \tag{5.4a}$$

to eliminate R_{II} yields the following steady state equations for C^* and C:

$$\alpha - k_1 R_I^0 D - \beta C^* - \gamma C = 0 \ , \quad k_2 C^* = k_{-2} C \ . \tag{5.4b, c}$$

Eliminating C^* in the above equations, and solving for C, we can write (5.3a) entirely in terms of D:

$$\frac{dD}{dt} = \frac{k k_1 R_I^0 (R_{II}^0 - D)}{\beta K_{-2} + \gamma} \ , \tag{5.5}$$

where $K_{-2} = k_{-2}/k_2$. We note that as $t \to \infty$, $D \to R_{II}^0$ as expected. Moreover,

$$\frac{k_1 R_I^0}{\beta K_{-2} + \gamma} = \frac{1}{1 + K_{-2}[1 + (K_{-1}/R_I^0)]} < 1 \ , \tag{5.6}$$

where $K_{-1} = k_{-1}/k_1$. Thus the effective rate constant for the slow $C \longrightarrow D$ transformation is smaller than k, owing to the slow decrease of C from the "steady state" value that is obtained when the $C \longrightarrow D$ transformation is neglected.

From a methodological point of view we have provided an example in which the QSSA approach easily extends to a case where there are three time domains: a fast transient, an intermediate transient, and a final slow and irreversible transition.

We reemphasize here certain aspects of matters discussed by Segel (1988) and Segel and Slemrod (1989). (i) In (4.1) we introduced dimensionless variables in an arbitrary manner, merely in order to reduce the number of parameters that appeared. If required, we are now in a position to introduce properly scaled dimensionless variables and thereby to set up expansion procedures that can provide more accurate answers. (ii) In verifying the various conditions that have been derived, we employed PB parameter estimates that were obtained using the very QSSA whose legitimacy we are testing. This is not circular reasoning; what we are doing is performing a consistency check. Lack of consistency is alarming; consistency means that all is well – unless the problem is ill-conditioned (Lin and Segel, 1988). (iii) Two conditions must be separately analyzed to determine the suitability of the QSSA: that the fast time scale is indeed small compared to the slow time scale and that the slow variable changes only negligibly during the fast transient. Generally, two separate conditions on the parameters are thereby derived. But for the examples considered here, no further restrictions are obtained by imposing the second condition.

The examples we have provided here and elsewhere show that once one "gets the hang of it," consistency checks are not all that difficult – at least for initial value problems of the type normally considered in biochemistry. Intuition or trial and error is used to decide which variables perhaps can be subject to a QSSA and then the procedure that we have illustrated is adopted to check for consistency.

To give a further indication that QSSA calculations can be successfully carried out in quite complex situations, we mention the model corresponding to the kinetic scheme

$$R_I + R_{II} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C^* \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C \ , \ R_{II} + M \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} R_M \ , \ R_I + R_M \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} C_M \overset{k_5}{\longrightarrow} C + M \ . \quad (5.7)$$

in which Rom binds to RNA II rather than the unstable complex. Scheme (5.7) can be expressed as four ordinary differential equations for C^*, R_M, C_M, and C. Once again, quasi-steady state assumptions are made for all variables except C. The counterpart of (4.4) now is a cubic in λ, but explicit estimates of the smallest root can be made when various reasonable assumptions are made on the parameters. In this manner, the various conditions that justify a QSSA can once again be derived. Perelson and Brendel (private communication) ultimately rejected this model as not in accord with experiment, so that provision of further detail is inappropriate.

Why bother with checking for consistency? A careful worker should certainly try to check whether the approximations that he or she has made are indeed consistent ones. Moreover, knowing the parameter ranges that permit consistent simplification of the problem may help in the design of easier or more revealing experiments.

Acknowledgements

This work was performed under the auspices of the U. S. Department of Energy and supported in part by the U.S. Israel Binational Science Foundation Grant 89-00146 and the Santa Fe Institute.

Caption for Figure

Fig. 1. Diagram of a ColE1 plasmid showing the regions that are transcribed into RNA I and RNA II. Notice that RNA I and RNA II are transcribed from opposite strands of the DNA and hence RNA I has a base sequence that is complementary to the 5′ end of RNA II. The binding of RNA I to RNA II prevents RNA II from acting as a primer for DNA synthesis. Rom, a 63 amino acid protein coded for upstream of the origin of replication, stabilizes the RNA I – RNA II interaction and enhances the ability of RNA I to inhibit plasmid replication.

References

Ausubel, F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. & Struhl, K., eds. (1987). *Current Protocols in Molecular Biology*, Vol. 1., Wiley-Interscience, New York.

Brendel, V. B. & Perelson, A. S. (1992). Kinetic model for the control of ColE1 plasmid copy number. (in preparation).

Brenner, M. & Tomizawa, J. (1991). Quantitation of ColE1-encoded replication elements. *Proc. natl. Acad. Sci. USA* **88**, 405–409.

Cesareni, G. & Banner, D.W. (1985). Regulation of plasmid copy number by complementary RNAs. *Trends Biochem. Sci.* **10**, 303–306.

Cesareni, G., Muessing, M. A. & Polisky, B. (1982). Control of ColE1 DNA replication: the rop gene product negatively affects transcription from the replication primer promoter. *Proc. Natl. Acad. Sci. USA* **79**, 6313–6317.

Dooley, T.P. & Polisky, B. (1987). Suppression of ColE1 RNA–RNA mismatch mutations *in vivo* by the ColE1 Rop protein. *Plasmid* **18**, 24–34.

Itoh, T. & Tomizawa, J. (1980). Formation of an RNA primer for initiation of replication of ColE1 DNA by ribonuclease H. *Proc. Natl. Acad. Sci. USA* **77**, 2450–2454.

Lin, C. C. & Segel, L. A. (1988). *Mathematics Applied to Deterministic Problems in the Natural Sciences* (2nd Ed.) SIAM Publications, Philadelphia.

Masukata, H. & Tomizawa, J. (1984). Effects of point mutations on formation and structure of the RNA primer for ColE1 DNA replication. *Cell* **36**, 513–522.

Masukata, H. & Tomizawa, J. (1986). Control of primer formation for ColE1 plasmid replication: Conformational change of the primer transcript. *Cell* **44**, 125–136.

Palsson, B. O. (1987). On the dynamics of the irreversible Michaelis-Menten reaction mechanism. *Chem Eng. Sci.* **42**, 447–458.

Perelson, A. S. & Brendel, V. (1989). Kinetics of complementary RNA-RNA interactions involved in plasmid ColE1 copy number control. *J. Mol. Biol.* **208**, 245–255.

Scott, J. R. (1984). Regulation of plasmid replication. *Microbiol. Rev.* **48**, 1–23.

Segel, L. A. (1988). On the validity of the steady state assumption of enzyme kinetics. *Bull. Math. Biol.* **50**, 579-593 (1988).

Segel, L. A. & Slemrod, M. (1989). The quasi-steady state assumption: a case study in perturbation. *SIAM Rev.* **31**, 446–477.

Selzer, G. & Tomizawa, J. (1982). Specific cleavage of the p15A primer precursor by ribonuclease H at the origin of DNA replication. *Proc. Natl. Acad. Sci. USA* **79**, 7082–7086.

Som, T. & Tomizawa, J. (1983). Regulatory regions of ColE1 that are involved in determination of plasmid copy number. *Proc. Natl. Acad. Sci. USA* **80**, 3232–3236.

Tamm, J. & Polisky, B. (1985). Characterization of the ColE1 primer-RNA1 complex: Analysis of a domain of ColE1 RNA1 necessary for its interaction with primer RNA. *Proc. Natl. Acad. Sci. USA* **82**, 2257–2261.

Tomizawa, J. (1984). Control of ColE1 plasmid replication: The process of binding of RNA I to the primer transcript. *Cell* **38**, 861–870.

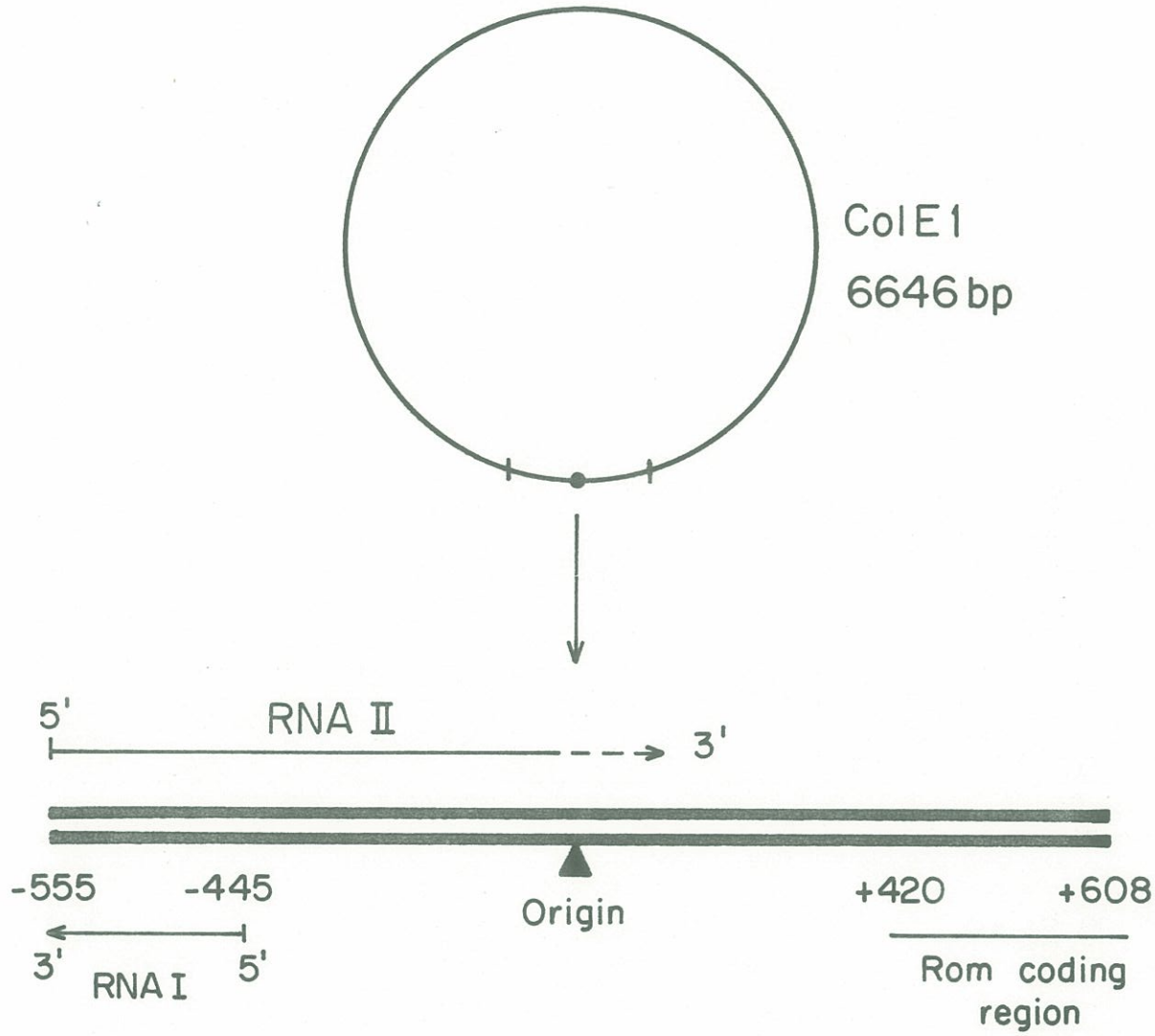
Tomizawa, J. (1985). Control of ColE1 plasmid replication: Initial interaction of RNA I and the primer transcript is reversible. *Cell* **40**, 527–537.

Tomizawa, J. (1986). Control of ColE1 plasmid replication: Binding of RNA I to RNA II and inhibition of primer formation. *Cell* **47**, 89–97.

Tomizawa, J. & Itoh, T. (1981). Plasmid ColE1 incompatibility determined by interaction of RNA I with primer transcript. *Proc. Natl. Acad. Sci. USA* **78**, 6096–6100.

Tomizawa, J. & Som, T. (1984). Control of ColE1 plasmid replication: Enhancement of binding of RNA I to primer transcript by the Rom protein. *Cell* **38**, 871–878.

Twigg, A. J. & Sherratt, D. (1980). Trans-complementable copy-number mutants of ColE1. *Nature* **283**, 216–218.


ColE1
6646 bp
5'
RNA II
3'
-555
-445
Origin
+420
+608
3'
RNA I
5'
Rom coding
region