Requirements for Rapid Plasmid ColE1 Copy Number Adjustments: 
A Mathematical Model of Inhibition Modes and RNA Turnover Rates

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The random distribution of ColE1 plasmids between the daughter cells at cell division introduces large copy number variations. Statistic variation associated with limited copy number in single cells also causes fluctuations to emerge spontaneously during the cell cycle. Efficient replication control out of steady state is therefore important to tame such stochastic effects of small numbers. In the present model, the dynamic features of copy number control are divided into two parts: first, how sharply the replication frequency per plasmid responds to changes in the concentration of the plasmid-coded inhibitor, RNA I, and second, how tightly RNA I and plasmid concentrations are coupled. Single (hyperbolic)- and multiple (exponential)-step inhibition mechanisms are compared out of steady state and it is shown how the response in replication frequency depends on the mode of inhibition. For both mechanisms, sensitivity of inhibition is “bought” at the expense of a rapid turnover of a replication preprimer, RNA II. Conventional, single-step, inhibition kinetics gives a sloppy replication control even at high RNA II turnover rates, whereas multiple-step inhibition has the potential of working with unlimited precision. When plasmid concentration changes rapidly, RNA I must be degraded rapidly to be “up to date” with the change. Adjustment to steady state is drastically impaired when the turnover rate constants of RNA I decrease below certain thresholds, but is basically unaffected for a corresponding increase. Several features of copy number control that are shown to be crucial for the understanding of ColE1-type plasmids still remain to be experimentally characterized. It is shown how steady-state properties reflect dynamics at the heart of regulation and therefore can be used to discriminate between fundamentally different copy number control mechanisms. The experimental tests of the predictions made require carefully planned assays, and some suggestions for suitable experiments arise naturally from the present work. It is also discussed how the presence of the Rom protein may affect dynamic qualities of copy number control.

Key Words: ColE1; plasmid; regulation; RNA; replication.

Replication of ColE1-type plasmids has been extensively studied both experimentally and theoretically and used as a model system for how replicons in general can be stably maintained in a growing cell population. ColE1 copy number control depends on the inhibition of a cis-acting RNA replication primer precursor (RNA II) by a trans-acting antisense RNA (RNA I) (Tomizawa and Itoh, 1981; Tomizawa and Cesareni, 1981). Primer formation is sensitive to RNA I attack when transcription proceeds through a particular inhibition window, approximately extending from base 110 to base 360, in the coding region for RNA II (Tomizawa, 1986). If the RNAs form a reversible initial “kissing” complex (Tomizawa, 1990a) that is converted into a stable duplex during transcription in this window, conformational changes in RNA II are triggered and the succeeding plasmid replication is inhibited. A small plasmid-encoded polypeptide, the Rom protein (RNA one modulator), stabilizes the
RNA I–RNA II interaction, increasing the probability of duplex formation from an initial “kissing” complex (Cesareni et al., 1982; Lacatena et al., 1984; Tomizawa and Som, 1984; Tomizawa, 1990b). If RNA I does not bind to RNA II in the inhibition window, RNA II can form a stable DNA–RNA hybrid at the ori. The RNA II part of the hybrid is subsequently cleaved by RNase H, yielding a mature replication primer for DNA polymerase I (Ito and Tomizawa, 1980). RNA I can bind to RNA II also after the inhibition window, but this does not prevent primer formation (Tomizawa, 1986).

Performance of replication control can be logically structured into two parts: how sharply the replication frequency responds to changes in RNA I concentration (the sensitivity of inhibition) and how strictly RNA I and plasmid concentrations are proportional. The present study shows how the replication frequency in situations out of steady state depends on the statistical variation in the time spent in the inhibition window, the RNA I concentration, and the initiation rate of RNA II transcription. The demand for a strict proportionality between RNA I and plasmid concentrations to achieve efficient control becomes sharper the higher the sensitivity of inhibition and it is also shown how the proportionality is only determined by the degradation rate of RNA I.

In an ideal case, replication should be negligible when plasmid concentration is higher and very frequent when it is lower than steady state. The molecular prerequisites for such high-sensitivity plasmid replication exist for ColE1 (Ehrenberg, 1996). Some features of this type of hypersensitive control using multiple-step inhibition kinetics were modeled by Ehrenberg, assuming that RNA I and plasmid concentrations are perfectly proportional, but it was pointed out that such an ideal coupling requires an infinite degradation rate of RNA I and, thus, infinite energy consumption. The present work removes this simplifying assumption and introduces radically new features.

The random distribution of plasmids between the two daughter cells at cell division that characterizes wild-type ColE1 (Summers and Sherratt, 1984) introduces large variation in copy number at the beginning of the cell cycle. Deviations from steady state also emerge spontaneously throughout the cell cycle due to statistical variation of a small number of molecules. This means that copy number control in individual cells is characterized by adjustment to steady state, even if the plasmid concentration of the population as a whole is at steady state. The capacity to correct deviations in plasmid concentration before the next cell division occurs has a large impact on the stability of plasmid maintenance (Paulsson and Ehrenberg, 1998). The study of replication out of steady state is thus of great importance to the understanding of plasmid biology (Summers, 1996). Many aspects of control cannot be analyzed without taking stochastic effects of single cells into account (Rosenfeld and Grover, 1993; Ehrenberg, 1996; Paulsson and Ehrenberg, 1998), but there are others that are difficult to treat without a simplified macroscopic approach (as the present work) that disregards such variation.

Various mathematical models have been proposed to explain the kinetics of regulation (Ataai and Shuler, 1986; Bremer and Lin-Chao, 1986; Keasling and Palsson, 1989a; Brenner and Tomizawa, 1989; Brendel and Perelson, 1993; Ehrenberg, 1996) but these often put emphasis on steady-state plasmid concentrations (Brenner and Tomizawa, 1989; Brendel and Perelson, 1993) rather than on non-steady-state replication. We show how steady-state properties are reflections of dynamics at the heart of replication control and thus can be used experimentally to discriminate between different possible dynamics of copy number control.

**KINETICS OF INHIBITION**

Formation of a replication primer can only be inhibited by RNA I during transcription in a window from base 110 to base 360 in the coding region for RNA II (Tomizawa, 1986). Before this window, the conformation of RNA
II does not allow formation of an initial RNA I:RNA II ‘‘kissing’’ complex, and after base 360, RNA I binding cannot effectively prevent primer formation. If primer formation is not inhibited, RNA II can form an RNA:DNA hybrid and initiate replication, but if it forms a stable duplex with RNA I, transcription proceeds beyond the ori and RNA II is released (Fig. 1). Not every mature primer can initiate replication successfully. To account for this fact we include the constant probability, \( \rho \), that an RNA II transcript that avoids inhibition initiates plasmid replication\(^1\) (Fig. 1 and Table 1) (Itoh and Tomizawa, 1980; Tomizawa and Itoh, 1981; Masukata and Tomizawa, 1986).

A brief recapitulation of multiple-step inhibition (Ehrenberg, 1996) will help to clarify the new developments in the present work.

When the RNA polymerase is at base \( x \) in the inhibition window either it can transcribe base \( x \) and proceed to \( x + 1 \) with rate constant \( k_u \) without RNA I:RNA II duplex formation, or duplex formation occurs with effective rate constant \( k_{ad} \cdot R_I \) (Tomizawa, 1986). Here \( k_{ad} \) is the effective association rate constant for the initial complex formation between RNA I and an RNA II of length \( x \), and \( R_I \) is the concentration of free RNA I (Fig. 2). The probability that an initial RNA I:RNA II complex is converted to a stable duplex is included in \( k_{ad} \), and is assumed to be independent of plasmid concentration, but may in fact play an important role in the dynamics of copy number control via the action of the Rom protein (see Discussion). The probability that primer formation is not inhibited at base \( x \) is therefore

\[
P^x = \frac{k_u}{k_u + k_{ad} \cdot R_I} = \frac{1}{1 + k_{ad} \cdot R_I/k_u},
\]

where \( \tau_u = 1/k_u \) is the average time that the polymerase stays in position \( x \).

The probability \( Q_0 \) that primer formation is not inhibited at all is the product of the probabilities that it is not inhibited at any of the single steps of the window:

\[
Q_0 = \prod_{x=110}^{360} P^x = \prod_{x=110}^{360} \frac{1}{1 + k_{ad} \cdot \tau_u \cdot R_I}. \quad [2]
\]

Normally, one would expect that some base transcriptions are on average more time consuming than others, so that the total average time spent in the inhibition window, \( \tau_u = \sum_{x=110}^{360} \tau_u^x \), can be dominated by a few transcription pauses. It has also been shown in vitro that the association rate constant \( k_{ad} \) is very different for different lengths of RNA II (Tomizawa, 1986). Throughout this analysis, we assume that contributions to \( \tau_u \) from base transcriptions other than \( n \) identical pause sites can be neglected and that \( k_{ad} \) is constant and equal to \( k_{ad} \) at all these sites. These conditions amend clarity notably without significantly reducing the generality of description. The average time it takes to transcribe one of these \( n \) bases, \( \tau_{un} \), is then given by

\[
\tau_{un} = \frac{\tau_u}{n}. \quad [3]
\]

The probability that a particular replication priming is not inhibited by RNA I follows directly from Eqs. [2] and [3] and is given by

\[
Q_0 = \left( \frac{1}{1 + k_{ad} \cdot \tau_u \cdot R_I/n} \right)^n = \left( \frac{1}{1 + R_I/(n \cdot K_I)} \right)^n, \quad [4]
\]

where \( K_I \) is defined as

\[
K_I = \frac{1}{k_{ad} \cdot \tau_u}. \quad [5]
\]

When \( n = 1 \), \( Q_0 \) is hyperbolic.\(^2\)

\(^1\) In the model of Ehrenberg (1996), \( \rho \) is included in \( k_u \) (the rate constant for RNA II transcription), but since the present analysis takes finite RNA I turnover rates into account, it is necessary to treat \( \rho \) separately from \( k_u \).

\(^2\) The same type of inhibition mechanism as for plasmid R1 (Nordström, 1985).
FIG. 1. Schematic overview of the replication priming process of plasmid ColE1. Both the preprimer RNA II (bases 0–555) and the inhibitor RNA I (bases 2–110 of the opposite strand) are constitutively transcribed. RNA I can bind to RNA II and form a stable duplex. If binding occurs during RNA II transcription in an "inhibition window," stretching roughly from base 110 to base 360 in the RNA II gene, conformational changes in RNA II are triggered and subsequent primer formation is inhibited. Before base 110, RNA I binds very inefficiently to RNA II, and after base 360, RNA I can still bind, but has basically no effect on primer formation. In the absence of inhibition, RNA II can form an RNA–DNA hybrid at the ori (base 555). The hybrid is then cleaved by RNase H, yielding a mature primer for DNA polymerase I.

\[ Q_0 = \frac{1}{1 + R_I/K_I} \]  

[6] \[ Q_0 = \lim_{n \to \infty} \left( \frac{1}{1 + R_I/(n \cdot K_I)} \right)^n = e^{-R_I/K_I} \]  

[7]

and in the limit when \( n \) goes to infinity, \( Q_0 \) is exponential (Ehrenberg, 1996) and given by When there are 250 equal steps in the inhibition window, a change in RNA I concentration that
### TABLE 1

**Definition of Parameters Used**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y$</td>
<td>Plasmid concentration</td>
</tr>
<tr>
<td>$\bar{y}$</td>
<td>Steady-state plasmid concentration</td>
</tr>
<tr>
<td>$\Delta y$</td>
<td>Deviation from steady state in plasmid concentration</td>
</tr>
<tr>
<td>$R_I$</td>
<td>RNA I concentration</td>
</tr>
<tr>
<td>$\bar{R}_I$</td>
<td>Steady-state RNA I concentration</td>
</tr>
<tr>
<td>$\Delta R_I$</td>
<td>Deviation from steady state in RNA I concentration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rate constant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{x}^{tr}$</td>
<td>Transcription rate constant at position $x$ in the RNA II coding region</td>
</tr>
<tr>
<td>$k_{al}$</td>
<td>Effective association rate constant of RNA I to RNA II at one of $n$ identical pause sites in the RNA II coding region</td>
</tr>
<tr>
<td>$k_{ii}$</td>
<td>Initiation rate constant of RNA II transcription</td>
</tr>
<tr>
<td>$k_{n}$</td>
<td>Growth rate constant of the host cell</td>
</tr>
<tr>
<td>$\rho \cdot k_{al} / k_{ii}$</td>
<td>Number of RNA II transcriptions per plasmid and cell cycle multiplied by the probability that a mature primer induces plasmid replication</td>
</tr>
<tr>
<td>$k_{i}$</td>
<td>Synthesis rate constant of RNA I transcription</td>
</tr>
<tr>
<td>$e_i$</td>
<td>Degradation rate constant of RNA I</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Decrease rate constant of free RNA I due to active degradation and dilution</td>
</tr>
<tr>
<td>$\beta$</td>
<td>“Net” synthesis rate constant of RNA I</td>
</tr>
<tr>
<td>$k_{adj}$</td>
<td>Adjustment rate constant for deviations in plasmid concentration</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\rho'$</td>
<td>Probability that a mature primer finally induces plasmid replication</td>
</tr>
<tr>
<td>$P$</td>
<td>Probability that RNA II does not bind RNA I during transcription of base $x$ in the RNA II coding region</td>
</tr>
<tr>
<td>$Q_0$</td>
<td>Probability that replication priming by RNA II is not inhibited by RNA I</td>
</tr>
<tr>
<td>$\tau_x$</td>
<td>Average time that RNA polymerase stays at base $x$ in the RNA II coding region</td>
</tr>
<tr>
<td>$\tau_u$</td>
<td>Average time that replication priming by RNA II is sensitive to inhibition by RNA I</td>
</tr>
<tr>
<td>$n$</td>
<td>Number of dominating pause sites in the transcription of the RNA II coding region</td>
</tr>
<tr>
<td>$\tau_{uu}$</td>
<td>Average time that RNA polymerase stays at a pause site when there are $n$ identical pause sites in the inhibition window</td>
</tr>
<tr>
<td>$K_I$</td>
<td>Inhibition constant for RNA I:RNA II complex formation</td>
</tr>
</tbody>
</table>

Reduces the probability that priming is not inhibited at a single step to 99% of its previous value reduces the probability that priming is not inhibited at any of the 250 steps to $0.99^{250} = 8\%$ of its previous value. Multiple-step inhibition can thus result in a very sharp response in $Q_0$ to moderate changes in RNA I concentration.

Another way to understand the difference between hyperbolic and exponential inhibition is to inspect the time that replication priming by RNA II is susceptible to inhibition. If there is a single rate-limiting step in the inhibition window, this time will have large statistical variation, leading to a sloppy replication control (hyperbolic, Eq. [6]). When there are very many rate-limiting steps, statistical variation in the inhibition time is negligible, making control much sharper (exponential, Eq. [7]).

Experiments indicate that the inhibition window is not precisely defined from base 110 to base 360 in the coding region for RNA II, that different bases are transcribed at different rates, and that the effective association rate constant between RNA I and RNA II is different for different lengths of RNA II (Tomizawa, 1986). However, $Q_0$ must always be confined between hyperbolic (Eq. [6]) and exponential (Eq. [7]) inhibition, corresponding to maximal and minimal “randomization” of the time spent in the inhibition window. If the...

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3 It will be an exponentially distributed stochastic variable.
**FIG. 2.** Kinetics in the inhibition window. RNA II is sensitive to RNA I binding at many steps during transcription in the inhibition window (bases 110–360). The probability that inhibition occurs at a certain base depends both on the transcription rate \(k_{tr}\) and on the rate of RNA I binding \(k_{aI}R_I\) at that base. The overall behavior of inhibition is determined by the number of rate-limiting steps in this window.

overall inhibition mechanism basically depends on one rate-limiting step, inhibition is essentially hyperbolic, and if there are many rate-limiting steps, inhibition is essentially exponential.

Both Eqs. [6] and [7] already exist in the literature, and for a long time it remained unclear (Brendel and Perelson, 1993; Merlin and Polisky, 1995) how there can be such different expressions for \(Q_0\). Brenner and Tomizawa (1991) assumed Eq. [7] without giving any explicit rationale, whereas other authors have used an approach similar to ours to derive the conventional, hyperbolic expression (Eq. [6]) (Bremer and Lin-Chao, 1986; Keasling and Palsson, 1989a; Brendel and Perelson, 1993).

Ehrenberg (1996) explained the differences between Eqs. [6] and [7] and showed how higher segregational stability could be obtained with exponential inhibition in the hypothetical situation that plasmids are equipartitioned between the daughter cells and that plasmid and RNA I concentrations are perfectly proportional.

It is still not known whether ColE1 uses exponential or hyperbolic inhibition. This is of crucial importance to the understanding of copy number control, and its experimental characterization deserves high priority (see Discussion).

**NONLINEAR MODEL**

*Differential Equations*

A convenient starting point for a macroscopic description of ColE1 replication control is to formulate differential equations for plasmid \(y\) and RNA I \(R_I\) concentrations:

\[
\frac{dy}{dt} = k_{II}Q_0\rho y - k_{III}y
\]

\[
\frac{dR_I}{dt} = (k_I - k_{II})y - (c_1 + k_{II})R_I. \tag{8}
\]

The change in plasmid concentration, \(dy/dt\), is determined by the replication rate per plasmid, \(k_{II}Q_0\rho\) (Table 1, Fig. 1), and \(k_{III}\), the growth rate of the host cell since all components in exponentially growing cells are subjects to continuous dilution (Ehrenberg and Kurland, 1984).

Plasmids are responsible for RNA I synthesis with rate \(k_I\cdot y\) and RNA I degradation due to RNA I–RNA II duplex formation\(^4\) with rate \(k_{III}\cdot y\).

Free RNA I is also actively degraded with

\(^4\) This assumes that every RNA II eventually binds an RNA I (during or after the inhibition window) and experiments suggest this to be the case (Tomizawa, 1986).
rate $\epsilon_1 \cdot R_1$ and diluted with rate $k_{II} \cdot R_1$. There are many steps in RNA I degradation, but intermediate forms of RNA I seem to be rapidly degraded and cannot inhibit primer formation significantly (Tomizawa, 1984; Tamm and Polisky, 1985; Lin-Chao and Cohen, 1991). They are therefore assumed not to take part in replication control and RNA I will be regarded as a single species where $\epsilon_1$ is the rate constant for the first step of its degradation.

The following notations are convenient:

$$
\epsilon_1 + k_{II} = \alpha, \tag{9}
$$

$$
k_1 - k_{II} = \beta, \tag{10}
$$

so that $\beta$ is the ‘‘net’’ synthesis rate of RNA I per unit of plasmid concentration.

Brendel and Perelson (1993) included 10 different concentration variables in their analysis. However, under very broad (and realistic) conditions, only concentrations $\gamma$ and $R_I$ are important. For the hyperbolic mode of inhibition, the expression for steady-state plasmid concentration in our analysis is identical to theirs.

**Steady-State Expressions**

The system of differential equations [8] can be used to obtain steady-state levels of RNA I and plasmid concentrations (Eqs. [A1]–[A4]) by setting $dy/dt$ and $dR_I/dt$ at zero:

$$
\bar{R}_I = \left( \rho \cdot \frac{k_{II}}{k_{II}} - 1 \right) \cdot K_I \tag{11}
$$

$$
\gamma = \frac{\alpha}{\beta} \left( \rho \cdot \frac{k_{II}}{k_{II}} - 1 \right) \cdot K_I \tag{12}
$$

for the hyperbolic and exponential modes of inhibition, respectively.

When $k_{II}$ is greater than $k_I$ there is no steady state ($\beta < 0$) and inhibition is insufficient to regulate copy number. This causes ‘‘runaway’’ replication (Brendel and Perelson, 1993) that eventually prevents the host cell from growing. However, under normal in vivo conditions $k_I$ is much larger than $k_{II}$ (Cesareni et al., 1984; Lin-Chao and Bremer, 1986, 1987; Brenner and Tomizawa, 1991), so that the decrease in RNA I due to duplex formation is negligible.

According to Merlin and Polisky (1995), the Brenner and Tomizawa (1991) model, unlike the Brendel and Perelson (1993) model, has no predictive power since it ‘‘merely summarizes self-consistent relationships.’’ This assertion is incorrect and the difference between their predictions depends instead on different assumptions about the mechanism of inhibition as explained above.

**Adjustment to Steady State**

When RNA I and plasmid concentrations are proportional, an efficient regulation depends only on how sharply the probability of inhibition of primer formation responds to changes from steady state in RNA I concentration. This is determined by the mechanism of inhibition (hyperbolic or exponential) and $\bar{R}_I/K_I$ (Eqs. [6] and [7]). As can be seen in Eqs. [11] and [12], this ratio is determined solely by $\rho \cdot k_{II}/k_{II}$, since any change in $K_I$ will result in a corresponding change in $\bar{R}_I$, leaving $\bar{R}_I/K_I$ unaltered. Accordingly, $K_I$ (Eq. [5]) determines steady-state concentrations, but has no effect on the dynamic properties of copy number control. The important parameter $\rho \cdot k_{II}/k_{II}$ corresponds to the number of RNA II transcriptions per plasmid and cell cycle, $k_{II}/k_{II}$.

\footnote{When Rom has the dynamic effect that was outlined by Ehrenberg (1996), Rom concentration should be included as a third concentration variable. For the set of rate constants used by Brendel and Perelson it is not necessary, however.}

\footnote{In a newborn cell.}
FIG. 3. Average number of replications per plasmid and cell cycle as a function of the normalized RNA I concentration for hyperbolic (Eq. [6]) and exponential (Eq. [7]) inhibition mechanisms. Both curves are obtained for $\rho \cdot k_d/k_H = 10$. In the upper right region plasmids replicate more than once per copy and cell cycle for elevated RNA I concentrations, leading to runaway replication, and in the lower left region, plasmids replicate less than once per copy and cell cycle for low RNA I concentrations and are lost.

multiplied by $\rho$, the probability that a primer that is not inhibited really initiates replication. For large values of $\rho \cdot k_d/k_H$ and exponential mode of inhibition, $Q_0$ responds very sharply to changes in RNA I concentration (Fig. 3). In contrast, for the hyperbolic mode of inhibition $Q_0$ becomes inversely proportional to plasmid concentration (Fig. 3) in the limit when $\rho \cdot k_d/k_H \to \infty$, making the mechanism less precise. This means that the total replication rate is constant, irrespective of plasmid concentration, equivalent to the ‘‘+n’’ model of plasmid R1 replication control (Nordström and Aagaard-Hansen, 1984; Nordström et al., 1984). The change in plasmid concentration is then given by

$$\frac{dy}{dt} = k_H \cdot (y - y)$$  \hspace{1cm} [13]  

both above and below steady state (Eq. [A5]).

Exponential and hyperbolic inhibition modes not only differ in the limit $\rho \cdot k_d/k_H \to \infty$. Although both mechanisms become increasingly sensitive with increasing values of $\rho \cdot k_d/k_H$, exponential inhibition always results in more sensitive control than hyperbolic inhibition (Fig. 4). Efficient copy number control depends on a good inhibition mechanism (exponential rather than hyperbolic) but for a given mechanism, quality can be bought at the expense of rapid RNA II turnover and hence increased metabolic load for the host cell. As
shown above, an inefficient mechanism, like hyperbolic inhibition, requires high RNA II turnover to obtain even rather “sloppy” control (Eq. [13], Figs. 3 and 4B).

It is clear that the response in average copy number to changes in \( \rho \cdot k_d/k_H \) directly reflects the sensitivity in replication control (Eqs. [6] and [7] together with [12] and [11]). Since a sharp copy number control can increase segregational stability remarkably (Summers, 1996; Paulsson and Ehrenberg, 1998), the experimentally unsettled question of how the steady-state plasmid concentration depends on \( \rho \cdot k_d/k_H \) is a key to the understanding of ColE1 regulation.

Smooth and rapid adjustment to steady state (Fig. 4) also requires an approximate proportionality between plasmid and RNA I concentrations that depends on rapid turnover of RNA I. To study effects of limited RNA I turnover and copy number control in the vicinity of steady state, a linearization of the nonlinear differential equations [8] is helpful.

A LINEARIZED MODEL FOR SMALL DEVIATIONS FROM STEADY STATE

Adjustment Rates

The system of differential equations [8] can be linearized in the vicinity of steady state using first-order Taylor expansions. Deviations from steady state in plasmid and RNA I concentrations are defined as \( \Delta y = y - \bar{y} \) and \( \Delta R_I = R_I - \bar{R}_I \), respectively. A linearization (Equations [A6]–[A14]) of system [8] yields

\[
\frac{d\Delta y}{dt} = -k_{adj} \cdot \frac{\alpha}{\beta} \cdot \Delta R_I
\]

\[
\frac{d\Delta R_I}{dt} = \beta \cdot \Delta y - \alpha \cdot \Delta R_I,
\]

where the compound rate constant \( k_{adj} \) (Fig. 5) depends on the mechanism of inhibition (Eq. [A13]) and \( \rho \cdot k_d/k_H \), like a linearized counterpart to \( Q_o \) (Eq. [2]). Hyperbolic and exponential modes of inhibition correspond to...
FIG. 5. The normalized adjustment rate constant $k_{adj}/k_H$ (Eqs. [15] and [16]) in the linearized system [14] as a function of $\rho \cdot k_{adj}/k_H$. (A) and (B) contain the same data illustrated in linear and logarithmic scale, respectively.

$$k_{adj} = k_H \cdot \left(1 - \frac{k_H}{\rho \cdot k_H}\right).$$  \[15\]

and

$$\lim_{n \to \infty} k_{adj} = k_H \cdot \ln\left(\frac{\rho \cdot k_H}{k_H}\right).$$  \[16\]

respectively. It is clear that $k_{adj} \approx k_H$ for hyperbolic inhibition where the equality holds only in the limit where $\rho \cdot k_{adj}/k_H \to \infty$. For exponential inhibition, $k_{adj}$ has no upper limit and increases logarithmically with $\rho \cdot k_{adj}/k_H$ (Fig. 5).

In the limit of infinite degradation rate of RNA I, plasmid and RNA I concentrations are perfectly proportional. In this idealized case, the deviation from steady state in plasmid concentration decreases exponentially with time (Eq. [A16]) as

$$\Delta y(t) = \Delta y(0) \cdot e^{-k_{adj} \cdot t}.$$  \[17\]

This relation demonstrates the importance of the rate constant $k_{adj}$ to how rapidly perturbations from steady state are adjusted.

Limited RNA I Degradation Rate

The adjustment to steady state illustrated in Fig. 4 depends on an approximate proportionality between plasmid and RNA I concentrations, which requires rapid degradation of RNA I. If RNA I concentration is not “up to date” with plasmid concentration, replication frequency is determined by obsolete “information” that may cause the system to over- and undershoot.

Solving the linearized system (Eq. [14]) analytically, calculating the eigenvalues (Eq. [A17]), discloses a parameter range where such oscillations occur:

$$\alpha < 4 \cdot k_{adj}.$$  \[18\]
The left side of Eq. [18], defined in Eq. [9], describes how rapidly RNA I concentration equilibrates to plasmid concentration (Eq. [14]), and the right side describes the rate of adjustment to steady state in plasmid concentration (Eqs. [14–16]). The minimum RNA I degradation rate that avoids oscillations is thus directly determined by how strongly the replication frequency responds to deviations from steady state in RNA I concentration, i.e., the sensitivity of inhibition. This feature is comparable to the problem of adjusting the temperature of an old shower. Turning the handle too fast (too high $k_{\text{adj}}$), acting only on the temperature of the water presently coming out (RNA I concentration), may cause you to overcompensate, rendering the water too cold instead of too hot. Pursuing this principle of regulation will leave you alternating between too hot and too cold showers (oscillating concentrations) that, even if they may connive a failing mental health (Foucault, 1965), are disastrous to regulation of temperature (plasmid concentration). Depending on how slowly the shower responds (the lag between plasmid and RNA I concentrations), there is a maximal adjustment rate that avoids oscillations. A hypersensitive copy number control adjusts deviations in plasmid concentration rapidly and must therefore be coupled to a high RNA I degradation rate to avoid oscillations (Eqs. [9] and [18]). The amplitude of the oscillations gradually decreases (the system is unconditionally stable) and finally the system reaches steady state (Fig. 6).

The present analysis clearly shows that the “lag” between plasmid and RNA I concentrations does not depend on both the rate constants for RNA I degradation and synthesis as previously argued, for instance, in the excellent book “The Biology of Plasmids” (Summers, 1996), but on the degradation rate constant alone.$^7$

Simulations show that the linearized system (Eq. [14]) very well predicts the $\alpha$ value (Eq. [18]) where oscillations emerge in the nonlinear system (Eq. [8]). The half-life of RNA I has been measured to be about 2 min for a cell generation time of 80 min (Brenner and Tomizawa, 1991).

### Limited RNA I Synthesis Rate

Experiments indicate that RNA I can bind to RNA II also after transcription of RNA II has passed base 360 (Tomizawa, 1986), but that this has little or no effect on primer formation. The fate of RNA II during transcription downstream of the ori, after release from RNA polymerase and after primer formation, is to our knowledge not determined. When RNA I binding is efficient also outside the inhibition window so that every RNA II eventually binds an RNA I, regardless of smaller fluctuations in RNA I concentration, then the rate of RNA I disappearance due to RNA I:RNA II duplex formation is $k_{\text{II}}$. In this case, the proportionality between plasmid and RNA I concentrations in the differential equations [8] is independent of $k_i$ (as long as $k_i > k_{\text{II}}$), so that $k_i$ will not influence the dynamics of copy number control in any way. However, the present macroscopic approach implicitly assumes that statistical variation in the number of plasmids and RNA I molecules can be disregarded. A complete microscopic analysis (Paulsson and Ehrenberg, 1998) has demonstrated that when $k_i$ is only slightly higher than $k_{\text{II}}$, there will inevitably be large statistical variation in the RNA I concentration that will result in slower adjustment to steady state. In such cases it is necessary to use microscopic models also to describe macroscopic phenomena.

The experimental evidence so far suggests that RNA II transcripts eventually form duplexes with RNA I (Tomizawa, 1986). However, the possibility cannot be excluded that the rate of disappearance of RNA I via duplex formation with RNA II depends on the current RNA I concentration. Under such conditions a new type of degeneracy could arise unless $k_i$ is not much larger than $k_{\text{II}}$. This phenomenon, which can be analyzed macroscopically, leads to a severe disproportionality between plasmid and RNA I concentrations (not shown).

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$^7$ Even in situations where the RNA I concentration should increase rapidly.
FIG. 6. Adjustment to steady state is illustrated by following the relative deviations from steady state in plasmid and RNA I concentrations when the degradation rate constant of RNA I is limited. The simulation is a numerical integration of the nonlinear system (Eq. [8]) for \( \rho \cdot k_d/k_{uI} = 10 \) and initial concentrations were set at 50% of the steady-state values.

DISCUSSION

Determinants of Efficiency of Copy Number Control

In the present analysis we have found that the efficiency of replication control out of steady state is determined by (1) the response in replication frequency to changes in RNA I concentration, depending on the mechanism of inhibition (exponential and hyperbolic mechanisms are highlighted) and the initiation rate of RNA II transcription per plasmid and cell cycle, \( k_d/k_{uI} \), multiplied by \( \rho \), the probability that a primer that avoids inhibition really induces replication; and (2) the relation between plasmid and RNA I concentrations. A strict proportionality requires that the degradation rate of RNA I is much higher than the rate of change in plasmid concentration close to steady state.

When RNA I and plasmid concentrations are strictly proportional throughout the cell cycle (rapid RNA I turnover) and \( \rho \cdot k_d/k_{uI} \) is very high (rapid RNA II turnover), exponential inhibition works almost like a replication switch button, so that replication is negligible when plasmid concentration is higher and very frequent when plasmid concentration is lower than at steady state (Figs. 3 and 4A). For hyperbolic inhibition the sensitivity of regulation is restricted in the sense that for very high \( \rho \cdot k_d/k_{uI} \), the replication frequency per plasmid becomes inversely proportional to plasmid concentration (Figs. 3 and 4B). This means that even if plasmid concentration is reduced to half of the steady-state level, the replication rate per plasmid can maximally be twice as high as the growth rate of the host cell (Eq. [13]). The difference between exponential and hyperbolic inhibition is very distinct also for limited \( \rho \cdot k_d/k_{uI} \) (Fig. 4). Thus, an exponential mechanism is always better than a hyperbolic one, and for a given mechanism, enhanced precision can always be gained by
a further increased RNA II transcription frequency.

Sensitive inhibition is not sufficient for precise control. Plasmid and RNA I concentrations must also be proportional. When active degradation of free RNA I is slow, there is inevitably a large lag between RNA I and plasmid concentrations that may lead to oscillations. The rate with which RNA I concentration must adjust to plasmid concentration (i.e., the degradation rate of RNA I) to avoid oscillative relaxation (Fig. 6) to steady state is determined by the adjustment rate to steady state in plasmid concentration (Eq. [18]). A sensitive regulation where plasmid concentration can change rapidly must thus keep a tight coupling between the concentrations by rapid RNA I degradation to achieve efficient control. Establishment phase analysis of ColE1 bacteriophage–plasmid hybrids (Merlin and Polisky, 1992) showed no oscillations but also showed that RNA I concentration was proportional to plasmid concentration in the entire establishment phase.

In vitro experiments suggest that basically every RNA I eventually binds an RNA II even if binding does not always inhibit primer formation efficiently (Tomizawa, 1986). This means that the proportionality between plasmid and RNA I concentrations does not at all depend on the transcription frequency of RNA I as long as RNA I is transcribed more frequently than RNA II. However, a stochastic analysis (Paulsson and Ehrenberg, 1998) reveals that there is inevitably a large statistical variation in the number of RNA I molecules in single cells unless RNA I is transcribed at a much higher frequency than RNA II.

The experimental characterization of the inhibition mode of ColE1 is nontrivial. It is probably impossible to accurately determine the number of rate-limiting steps of the inhibition process in vitro. The inhibition mode depends on the transcriptional pause pattern in the RNA II coding region, all effective association rate constants between RNA I and RNA II’s of different lengths (Tomizawa, 1986), and the effect that RNA I binding has on primer formation. However, there are other ways to characterize the inhibition mechanism.

**Experiments on Adjustment Rates to Steady State**

Experiments studying the establishment phase of bacteriophage–plasmid hybrids (Merlin and Polisky, 1992) could in principle be used to discriminate between different inhibition modes (exponential and hyperbolic). Figure 4 illustrates how the establishment rate is predicted to depend on \( \rho \cdot k_d / k_{III} \) and the inhibition mechanism, showing that careful data analysis may be required to separate exponential and hyperbolic inhibition for some values of \( \rho \cdot k_d / k_{III} \). It is therefore necessary that copy number adjustment be monitored all the way up to steady state with high accuracy. The only thing that can be deduced from Merlin and Polisky’s experimental data is that they are inconsistent with a simple hyperbolic mode of inhibition without a Rom effect (see below) as outlined by Ehrenberg (1996) or by Summers (1996). Merlin and Polisky also state that replication in the recovery phase does not depend on plasmid concentration but rather increases linearly with time. However, simulations show that such a conclusion cannot be drawn from their data that are, in this respect, consistent with many different mechanisms of inhibition.

Another way to study adjustment rates is to insert an additional coding region for RNA I with an inducible promoter. The steady-state plasmid concentration becomes lower when transcription of the inserted coding region is turned on, and goes back to normal when it is turned off again. The relaxation between the two different steady-state levels could then be used to discriminate between different models of inhibition. A minor complication is that an increased frequency of RNA I transcription per plasmid, \( k_{I} \), affects RNA I concentration only temporarily (Eqs. [11] and [12]) since the increase in RNA I concentration suppresses plasmid concentration proportionally so that the rate of RNA I transcription...
is restored. This means that plasmid and RNA I concentrations cannot be proportional in the initial phase of the relaxation experiment, reducing the adjustment rate to steady state for plasmids in this phase. Unambiguous interpretations of experimental data would then require that plasmid and RNA I concentrations are monitored simultaneously during the adjustment phase.

**Steady-State Experiments to Discriminate between Hyperbolic and Exponential Inhibition**

Sensitivity of inhibition is directly reflected in copy number responses to changes in $k_{II}/k_{II}$. For hyperbolic regulation, a twofold increase in $k_{II}$ results in at least a twofold increase in plasmid copy number (Eq. 11), while hypersensitive regulation (exponential inhibition and rapid RNA II turnover) implies basically no change in copy number by the same twofold increase in $k_{II}$ (Eq. 12). This way, steady-state properties can be used to understand dynamic properties at the heart of replication control.

The steady-state concentration of plasmids depends on many kinetic rate constants apart from $k_{II}/k_{II}$. A quantitative measurement of plasmid concentration that could reveal qualitative aspects of control can thus be very sensitive to unforeseen changes in other parameters. For instance, changes in the transcription rate of one DNA strand could have a major impact on the transcription rate of the corresponding antisense RNA from the other strand (Nordström and Uhlin, 1992). Genetic engineering to overexpress RNA II can thus cause a change in the RNA I synthesis rate with a radical impact on copy number (Eqs. 11 and 12). The ratio $k_{II}/k_{II}$ can also be changed by changing the growth rate of the host cell ($k_{II}$) with medium shifts (Bremer and Lin-Chao, 1986). However, many relevant rate constants are under physiological control (Bremer and Lin-Chao, 1986) so subsequent changes in copy number or in the adjustment rate to steady state can have many different plausible rationales.

One way to avoid such ambiguities is to use plasmid multimerization experiments. The recombination of two plasmids, resulting in a plasmid dimer, implies that a successful initiation of replication at any of the two (unaltered) ori will cause the entire dimer to replicate, but also that there will be two genes coding for RNA I. Assuming hyperbolic inhibition, Bremer and Lin-Chao (1986) predicted that these two effects would cancel each other so that the copy number for a dimer population would be more or less the same as for monomers (Fig. 7) and that the number of replication origins at steady state would be doubled. For the exponential mode of inhibition, the prediction would instead be that the copy number for dimers is about half compared with monomers (Fig. 7 and Eq. 12), so that the number of replication origins is more or less unchanged. Later, Chiang and Bremer (1988) showed experimentally that, in fact, the number of origins, and not the number of plasmid molecules, is more or less constant, supporting the assumption of exponential inhibition. Summers et al. (1993) argue that ColE1-type plasmids “count origins rather than independent plasmid molecules” and the present analysis shows that this depends on the number of rate-limiting steps in the inhibition process. We predict that the way of counting is a direct reflection of how the steady-state plasmid concentration depends on the transcription frequency of RNA II, so that a well-working single-step (hyperbolic) inhibition mechanism seems to be counting independent plasmid molecules while a well-working multiple-step (exponential) inhibition mechanism seems to be counting origins.

Summers et al. (1993) also reported that their experiments indicate a dimer copy number well below that of monomers but still greater than 50%. This is in perfect consistency with exponential inhibition and the experimentally measured values of $\rho \cdot k_{II}/k_{II}$ (Fig. 7).

To ensure that the effects of the inhibition window (exponential or hyperbolic inhibition) are separated from possible Rom effects, Rom should preferentially be overexpressed and at
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FIG. 7. Expected number of replication origins in a population consisting of dimers, ori$_{dimer}$, divided by the corresponding number for a monomer population, ori$_{monomer}$, as a function of $\rho k_H / k_H$. A proposed realistic region where $5 < \rho k_H / k_H < 100$ (Lin-Chao and Bremer, 1986; Brenner and Tomizawa, 1991) is shaded. The simulations were made under the assumption that the Rom protein is present in saturating concentrations so that effects of the inhibition window can be clearly separated from possible dynamic effects of Rom.

Why Is There a Rom Protein?

The capacity of RNA I to bind to RNA II (and inhibit primer formation if binding occurs in the inhibition window) is enhanced by the plasmid-encoded Rom protein (Cesareni et al., 1982; Som and Tomizawa, 1983; Tomizawa and Som, 1984). Initially, RNA I and RNA II form a reversible, “kissing” complex which can be converted to a stable duplex through a series of intermediates of differing stability (Perelson and Brendel, 1989; Tomizawa, 1990a). A single copy of Rom can bind to and stabilize the initial complex (Tomizawa, 1990b). The finding that the only effect of deleting the gene coding for Rom seems to be a two- to threefold increase in copy number (Twigg and Sherratt, 1980) motivated Brendel and Perelson (1993) to ask why evolution has provided ColE1 with a gene coding for Rom if this protein only modulates the steady-state copy number, since an increased transcription rate of RNA I would have exactly the same consequence.

One suggestion for a nontrivial function of Rom came from Ehrenberg (1996), who made the point that if Rom and plasmid concentrations are proportional throughout the cell cycle and if the probability that the initial RNA I–RNA II kissing complex forms a stable duplex is proportional to Rom concentration, then the probability of primer inhibition depends on the product of RNA I and Rom concentrations. As a consequence, the response in replication frequency to changes in plasmid concentration becomes sharper than if RNA I acted alone and this
significantly enhances precision in copy number control for exponential as well as hyperbolic inhibition.

Indirect support for this idea comes from the amino acid sequence of Rom. The second amino acid from the N terminal is a threonine, which at this position normally stabilizes ubiquitin binding, which could initialize a rapid degradation of the protein (Bachmair et al., 1986). Since Rom mRNA is very unstable⁸ (Brenner and Tomizawa, 1991), a rapid turnover of Rom would make its concentration close to proportional to the plasmid concentration, so that a “quadratic” response in the frequency of replication to changes in plasmid concentration could follow naturally. If the function of Rom only was to downregulate plasmid copy number one would not expect rapid degradation of Rom, since this would increase the metabolic burden of the host cell without any compensating benefit. One way to avoid interference of such a putative Rom effect in experiments to discriminate between hyperbolic and exponential inhibition would be to overexpress Rom so that Rom concentration is always saturating.

Another role of Rom could be to ensure a sufficiently large “dynamic range” for copy number control. This proposal is based on the assumption that the RNA I:RNA II interaction in the absence of Rom is too weak and the intrinsic rate of duplex formation too slow to ensure 100% inhibition of replication even at saturating concentrations of RNA I. If this is so, copy number control must necessarily be much less efficient⁹ than if inhibition is total at high RNA I concentrations. Furthermore, the advantage of exponential, in relation to hyperbolic, regulation for ColE1 cannot be implemented if the dynamic range of replication inhibition is small. The role for Rom could therefore be to make the probability of plasmid replication very close to zero at high RNA I concentrations, thereby making the dynamic range of the control system sufficiently large for efficient (hyperbolic as well as exponential) copy number regulation.

A third possibility for Rom function is that Rom could act as a backup system when plasmid concentration is greatly reduced (Summers, 1996) so that the replication frequency under normal conditions would be independent of small deviations in Rom concentration, but when it is reduced below a threshold, there is a sharp decline in the inhibition of replication and a corresponding increase in the replication frequency.

In the perspective of these different suggestions concerning Rom it would be of interest to find out experimentally whether or not Rom is rapidly degraded.

**CONCLUSIONS**

We have demonstrated how rapid adjustment of perturbations in plasmid concentration depends on a sensitive mechanism of inhibition and how efficiency is bought at the expense of rapid turnover of RNA I and RNA II. A hypersensitive copy number control increases segregational stability of plasmids greatly (Summers, 1996; Paulsson and Ehrenberg, 1998). However, the rapid turnover of the regulatory elements that is required for sensitivity imposes metabolic stress on the host cell. Since long-term stability in plasmid maintenance depends on both segregational stability and metabolic load (Proctor, 1994), efficiency of copy number control in terms of molecular mechanisms and the kinetic rate constants is necessary to understand the evolutionary strategy of plasmids. Many of the critical characteristics of ColE1 copy number control still await experimental characterization.

**APPENDIX**

**Steady State**

The differential equations (Eq. [8]) equal zero at steady state

⁸ An indication in itself that Rom might have this effect.

⁹ Since it implies an upper limit in $p \cdot k_{a1}k_{d1}$ to avoid runaway replication.
\[
\frac{dy}{dt} = (k_{II} \cdot \rho \cdot Q_0(R_1) - k_{II}) \cdot y = 0,
\]

\[
\frac{dR_1}{dt} = \beta \cdot \bar{y} - \alpha \cdot \bar{R}_1 = 0,
\]  \hspace{1cm} \text{[A1]}

where \( Q_0 \) is determined by \( n \), the number of rate-limiting steps in the inhibition window (Eq. [4]), as

\[
Q_0(R_1) = \frac{1}{(1 + R_1/(n \cdot K_1))^n}. \hspace{1cm} \text{[A2]}
\]

The first equation in [A1] then gives

\[
k_{II} \cdot \rho \cdot Q_0(R_1) = k_{II} \cdot \frac{\bar{R}_1}{K_1} = n \cdot \left( \left( \frac{\rho}{k_{II}} \cdot \frac{\bar{R}_1}{K_1} \right)^{1/n} - 1 \right), \hspace{1cm} \text{[A3]}
\]

and the other,

\[
\beta \cdot \bar{y} = \alpha \cdot \bar{R}_1 \Rightarrow \bar{y} = \frac{\alpha \cdot \bar{R}_1}{\beta}, \hspace{1cm} \text{[A4]}
\]

directly giving Eqs. [11] and [12] for hyperbolic \((n = 1)\) and exponential \((n \to \infty)\) inhibition, respectively.

**Maximal Adjustment Rate for Hyperbolic Inhibition**

For hyperbolic inhibition and \( \rho \cdot k_{II} / k_{III} \gg 1 \), Eqs. [8] and [11] give

\[
\frac{dy}{dt} = \left( \frac{k_{II} \cdot \rho}{1 + R_1 / k_{II}} - k_{II} \right) \cdot y
\]

\[
= \frac{k_{II} \cdot \rho \cdot y}{1 + (R_1 / \bar{R}_1) \cdot (k_{II} \cdot \rho / k_{III}) - k_{II} \cdot y \approx k_{II} \left( \frac{\bar{R}_1}{R_1} \cdot y - \frac{y}{R_1} \right) \hspace{1cm} \text{[A5]}
\]

Equation [13] is thus valid when RNA I and plasmid concentrations are strictly proportional throughout the cell cycle so that \( y/R_1 = \bar{y}/\bar{R}_1 \).

**Linearization**

The system of differential equations (Eq. [8]) can be linearized for small deviations from steady state, \( y = \Delta y + \bar{y} \) and \( R_1 = \Delta R_1 + \bar{R}_1 \). Since \( \bar{y} \) and \( \bar{R}_1 \) are constant, the derivatives are unchanged:

\[
\frac{dy}{dt} = \frac{d\Delta y}{dt},
\]

\[
\frac{dR_1}{dt} = \frac{d\Delta R_1}{dt}. \hspace{1cm} \text{[A6]}
\]

Using this in system [8] yields

\[
\frac{d\Delta y}{dt} = (k_{II} \cdot \rho \cdot Q_0(\bar{R}_1 + \Delta R_1) - k_{II}) \times (\bar{y} + \Delta y),
\]

\[
\frac{d\Delta R_1}{dt} = \beta \cdot (\bar{y} + \Delta y) - \alpha \cdot (\bar{R}_1 + \Delta R_1). \hspace{1cm} \text{[A7]}
\]

For small \( \Delta R_1 \), \( Q_0 \) can be approximated by a first-order Taylor expansion so that directly giving Eqs. [11] and [12] for hyperbolic \((n = 1)\) and exponential \((n \to \infty)\) inhibition, respectively.

**Maximal Adjustment Rate for Hyperbolic Inhibition**

\[
Q_0(\Delta R_1 + \bar{R}_1) = Q_0(\bar{R}_1) + Q_0'(\bar{R}_1) \cdot \Delta R_1, \hspace{1cm} \text{[A8]}
\]

where

\[
Q_0(\bar{R}_1) \cdot \Delta R_1 = -\frac{\Delta R_1}{\bar{R}_1} \cdot Q_0(\bar{R}_1) \cdot (Q_0(\bar{R}_1))^{1/n} \hspace{1cm} \text{[A9]}
\]

Equations [A3], [A8], and [A9] lead to

\[
k_{II} \cdot \rho \cdot Q_0(\Delta R_1 + \bar{R}_1)
\]

\[
\approx k_{II} - k_{II} \cdot \frac{\Delta R_1}{\bar{R}_1} \cdot (Q_0(\bar{R}_1))^{1/n} \hspace{1cm} \text{[A10]}
\]

Using this relation in the first differential equation of system [8] gives
\[
\frac{d\Delta y}{dt} = -k_H \left( \frac{k_H}{k_H \cdot \rho} \right)^{1/n} \times \frac{\Delta R_1}{K_1} \cdot (\bar{y} + \Delta y). \quad \text{[A11]}
\]

Under the assumption that deviations from steady state are small so that \( \Delta R_1 \cdot (\bar{y} + \Delta y) \approx \Delta R_1 \cdot \bar{y} \), Eqs. [A3], [A4], and [A11] lead to

\[
\frac{d\Delta y}{dt} = -k_H \cdot n \left( 1 - \left( \frac{k_H}{k_H \cdot \rho} \right)^{1/n} \right) \cdot \frac{\alpha}{\beta} \cdot \Delta R_1, \quad \text{[A12]}
\]

where the following notation becomes convenient:

\[
k_{ad} = k_H \cdot n \left( 1 - \left( \frac{k_H}{k_H \cdot \rho} \right)^{1/n} \right), \quad \text{[A13]}
\]

yielding Eqs. [15] and [16] for hyperbolic and exponential inhibition, respectively.

For the second equation in system [8], [A4] directly gives

\[
\frac{d\Delta R_1}{dt} = \beta \cdot (\bar{y} + \Delta y) - \alpha \cdot (R_1 + \Delta R_1)
= \beta \cdot \Delta y - \alpha \cdot \Delta R_1, \quad \text{[A14]}
\]

so that the linearized system [14] is given. When \( \alpha \) is very large, the second linearized differential equation can be set at zero, since changes in \( \Delta R_1 \) are much faster than for \( \Delta y \), so that

\[
\frac{\alpha}{\beta} \Delta R_1 = \Delta y. \quad \text{[A15]}
\]

System [14] can then be reduced to

\[
\frac{d\Delta y}{dt} = -k_{ad} \cdot \Delta y. \quad \text{[A16]}
\]

To obtain inequality [18], the eigenvalues of Eq. [14] are calculated by standard methods:

\[
\lambda_{1,2} = -\frac{\alpha}{2} \left( 1 \pm \sqrt{1 - 4 \cdot \frac{k_{ad}}{\alpha}} \right). \quad \text{[A17]}
\]

The real part of the eigenvalues must thus always be negative so that the system is unconditionally stable in an asymptotic sense. The eigenvalues are complex and plasmid and RNA I concentrations have oscillations to steady state when inequality [18] is fulfilled.

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**REFERENCES**


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

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