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Quantitative Model of ColE1 Plasmid Copy Number Control

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Initiation of replication of the *Escherichia coli* plasmid ColE1 is inhibited by formation of a complex between a small plasmid RNA (RNA I) and the pre-primer for DNA synthesis (RNA II). Complex formation (and inhibition of replication) is enhanced by the plasmid-encoded Rom protein. The *in vitro* kinetics of complex formation were previously studied both experimentally and theoretically. The *in vivo* concentrations and half-lives of RNA I, RNA II and Rom protein have been measured recently. We present a dynamic model for the *in vivo* replication control mechanism that accounts for the measured concentration values. From the model we deduce a simple formula for the steady-state plasmid concentration. Our results agree with a previous simple steady-state analysis done by Brenner and Tomizawa, in that plasmid copy number is most strongly dependent on the per plasmid rate of RNA I synthesis. However, our model predicts other parameter dependencies that are not evident from or at variance with the previous analysis. Accordingly, we predict that plasmid copy number is greatly influenced by changes in the rate constant describing the formation of an initial unstable RNA I–RNA II complex, but is only slightly influenced by changes in the dissociation rate of this complex. Plasmid copy number per average cell volume is predicted to increase linearly with increases in the RNA II synthesis rate and with increases in the generation time of the host culture. Rom protein, which promotes conversion of the unstable RNA I–RNA II complex to a stable complex, serves to decrease copy number; however, its presence or absence does not seem to qualitatively alter the copy number control mechanism. Our model predicts the quantitative increase of plasmid copy number in *rom*$^-$ mutants. Several experiments are suggested to investigate the predictions of the model.

Keywords: ColE1; plasmid copy number; RNA I; RNA II; Rom (Rop) protein

1. Introduction

The factors and interactions involved in replication control of the ColE1 family of plasmids have been studied in great detail (for reviews, see Cesareni & Banner, 1985; Polisky, 1988; Kües & Stahl, 1989). Central to the regulatory mechanism are two complementary RNA transcripts, RNA I and RNA II, encoded upstream from the plasmid origin of replication. Some proportion of the RNA II transcripts that extend beyond the origin form a stable hybrid with the template DNA near the origin (Itoh & Tomizawa, 1980; Masukata & Tomizawa, 1984, 1986). RNase-H-mediated cleavage of some of the hybridized transcripts at the origin yields a primer for the initiation of DNA synthesis (Itoh & Tomizawa, 1980; Selzer & Tomizawa, 1982). RNA I can interact with RNA II to form an RNA I–RNA II complex that prevents RNA II from forming primer (Tomizawa & Itoh, 1981; Tomizawa, 1984). A 63 amino acid residue protein (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).

For inhibition to occur, RNA I must bind to the nascent RNA II chain before the RNA II transcript

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forms a hybrid with the template DNA (Tomizawa, 1986). The kinetics of the binding reaction are therefore crucial to the regulation of primer formation. The formation of a complex of RNA I and RNA II in the presence and absence of Rom protein has been studied *in vitro* for wild-type and mutant RNAs (Tomizawa, 1984, 1985; Tomizawa & Som, 1984; Dooley & Polisky, 1987). The formation of complex appears to follow a stepwise mechanism whereby initially RNA I and RNA II interact transiently via loop–loop interactions and subsequently base-pairing proceeds from the 5′-end of RNA I until RNA I is completely hybridized to the complementary sequence in RNA II (Tomizawa, 1984, 1990*a*).

Although a number of kinetic models have been presented for copy number control of ColE1 plasmids (Seo *et al.*, 1985; Bremer & Lin-Chao, 1986; Ataai & Shuler, 1986, 1987; Peretti & Bailey, 1987; Keasling & Palsson, 1989*a*,*b*; Brenner & Tomizawa, 1991), these models do not incorporate many recently discovered kinetic details such as the formation of an unstable complex between RNA I and RNA II. Here we develop a model of copy number control based on our previous analysis of the kinetics of RNA I–RNA II interaction *in vitro* (Perelson & Brendel, 1989). It is shown that incorporation of synthesis and turnover of DNA, RNA and protein, as well as bacterial growth into the model leads to a functional copy number control mechanism of the inhibitor dilution type (Pritchard *et al.*, 1969; Pritchard, 1984). The model can correctly account for the decrease in copy number in the presence of an intact *rom* gene as well as for the increase in plasmid concentration observed with diminished bacterial growth rate. Furthermore, the model gives a rationale for the changes in copy number seen with various mutant plasmids. The mathematical presentation of the model is given in the next section, followed by sections on model predictions and the mechanism of replication inhibition.

2. Kinetic Model of Replication Control

Figure 1 shows in schematic form the kinetic relationships between different molecular species involved in the control of plasmid ColE1 replication. The following variables are considered:

D concentration of free plasmid DNA.
D_{II}^{s} concentration of plasmid DNA complexed with an RNA II molecule of length 110 to 360 nucleotides.
D_{II}^{l} concentration of plasmid DNA complexed with an RNA II molecule of length greater than 360 nucleotides.
D_p concentration of replication-primed plasmid DNA.
D_c^* concentration of unstable complex of RNA I with plasmid-bound RNA II.
D_c concentration of stable complex of RNA I with plasmid-bound RNA II.
D_M concentration of an intermediate complex of RNA I with plasmid-bound RNA II and Rom protein.
R_I concentration of free RNA I.
R_{II} concentration of free RNA II.
M concentration of free Rom protein.

In modeling the various regulatory interactions we assume that RNA I and Rom protein are freely diffusing in the cytoplasm and are able to interact with any plasmid present. Such *trans* action of RNA I was demonstrated, for example, by Tomizawa (1985), who showed that RNA I of a heterologous coinfecting plasmid can interact with ColE1 RNA II.

Plasmid DNA is initially associated with the preprimer form of RNA II. For RNA I to prevent primer formation it must complex with D_{II}^{s}, the plasmid-bound RNA II transcript when it is between about 110 and 360 nucleotides long (Tomizawa, 1986). Some of the RNA II transcripts escape interaction with RNA I, elongate to D_{II}^{l}, hybridize with DNA at the origin of replication, and are processed by RNase H to form primer (D_p). The concentration and activity of RNase H will be assumed constant and incorporated into an effective rate constant, k_p, for primer formation from D_{II}^{l} (Fig. 1). Once a primer is formed, plasmid DNA is replicated with rate constant k_D and the RNA II primer is excised and released (Itoh & Tomizawa, 1980). RNA II transcripts that escape interaction with RNA I due to elongation and release are accounted for by the conversion of D_{II}^{l} back into D, with rate constant k_{-1}. Released RNA II is presumed to play no further role in replication control, but the concentration of free RNA II is kept track of in the model as one of the experimentally measurable quantities. Although our previous *in vitro* model (Perelson & Brendel, 1989) could be used to describe the kinetics of potential interactions between released RNA II transcripts and RNA I, we believe that such interactions are not important in copy number control and may be left out of the *in vivo* model without harm. The justification for this is that the number of RNA I molecules per cell is sufficiently large (Brenner & Tomizawa, 1991) that possible binding with released transcripts should not significantly decrease the pool of free RNA I. Similarly, free Rom protein is present at sufficiently high concentrations that it is justified to ignore its possible depletion by interaction with free RNA I–RNA II complexes (Brenner & Tomizawa, 1991).

The inhibitory processes are modeled as suggested by the *in vitro* binding kinetics (Perelson & Brendel, 1989): (i) formation of an unstable complex (D_c^*) between RNA I and plasmid DNA-bound RNA II of appropriate length (D_{II}^{s}); (ii) conversion of this unstable complex to a stable RNA I–RNA II complex (D_c); (iii) dissociation of stable complex from the DNA template. Dissociated stable complexes are partially double-stranded RNA and are assumed to be degraded without playing any

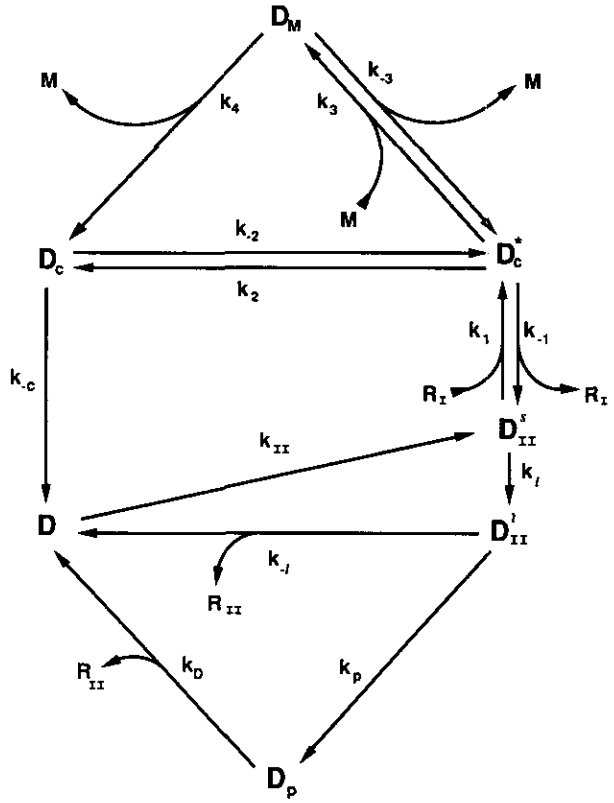

Figure 1. Kinetic scheme of the ColE1 replication control mechanism. Plasmid DNA occurs either in free form (D) or in association with RNA II transcripts (for short transcripts of 110 to 360 nucleotides, D_{II}^s; for longer transcripts that extend beyond the origin, D_{II}^l), with RNA II primer (D_p), or with bound complex (D_c^*, D_M, D_c). Short-length plasmid-bound RNA II (D_{II}^s) forms an unstable complex with RNA I (D_c^*). D_c^* converts directly or after association with Rom (M) protein (forming D_M) to stable complex D_c, which subsequently converts back to free DNA as the RNAs are released. The replication step is conversion of primed DNA (D_p) to free DNA (D). The conversions between the different states occur at the rates indicated. DNA-dependent synthesis of RNA I and Rom protein is not represented.

further role in the model. We assume that Rom protein binds to the unstable complex D_c^*, forming an intermediate complex (D_M) composed of RNA I, plasmid DNA-bound RNA II and Rom protein, which then rapidly converts into the stable RNA I–RNA II–DNA complex, D_c. Rom protein, by providing an alternative pathway for the formation of stable complex, makes the interaction of RNA I with RNA II more efficient. In the absence of Rom protein, a large fraction of RNA I–RNA II interactions are transient and are followed by rapid dissociation. In the presence of Rom protein, the unstable complex D_c^* is rapidly converted into a more stable form, D_M, and dissociation is thus prevented.

Other molecular reactions included in our model, but not depicted in Figure 1, are synthesis of RNA I and of Rom protein, at rates k_I and k_M per plasmid, respectively. We assume these rates to be constant, although they may be influenced by factors concomitant with high plasmid copy number. Degradation or decay of RNA I, RNA II and Rom protein is assumed to occur at rates ε_I, ε_{II}, and ε_M per molecule, respectively. RNA I degradation is known to be a stepwise process, involving: (1) an initial, rate-limiting RNase E-mediated endonucleolytic cleavage that removes the five 5′-terminal nucleotides; and (2) subsequent degradation of the RNase E cleavage product (called RNA I_{-5}) by an unidentified ribonuclease (Lin-Chao & Cohen, 1991). The parameter ε_I in our model represents the overall degradation rate constant of functional RNA I. This is essentially the rate constant of the first step in the pathway, as the second step is very rapid, and also the RNA I_{-5} has greatly reduced capacity to bind to RNA II (Tomizawa, 1984; Tamm & Polisky, 1985; Lin-Chao & Cohen, 1991).

Plasmid copy number is measured experimentally for cultures of host bacteria. The observed values may subsequently be converted to numbers per average cell volume or per cell mass (Brenner & Tomizawa, 1991; see Bremer & Lin-Chao (1986) for a discussion of appropriate choice of units). We shall consider the total cytoplasmic volume of the host culture as a homogeneous reaction volume (which is growing exponentially with rate constant μ due to individual cell growth and proliferation). We express concentrations in molarity, and we interpret numerical results scaled to units per cell as average values over all cells in the culture. For stochastic models of plasmid replication that study the effect of chance fluctuations at the single cell level on the segregation of plasmids among daughter cells at cell division and hence the distribution of plasmids in a population, see Novick & Hoppensteadt (1978), Ishii *et al.* (1978) and Seneta & Tavaré (1983).

Following standard mass action arguments applied to the reaction scheme displayed in Figure 1, the following differential equations are set forth to describe the *in vivo* kinetics of plasmid copy number control:

$$\frac{dD}{dt} = 2k_D D_p - (k_{II}+\mu)D + k_{-l}D_{II}^l + k_{-c}D_c \quad (1a)$$

$$\frac{dD_{II}^s}{dt} = k_{II}D - (k_l + k_1 R_I + \mu)D_{II}^s + k_{-1}D_c^* \quad (1b)$$

$$\frac{dD_{II}^l}{dt} = k_l D_{II}^s - (k_{-l} + k_p + \mu)D_{II}^l \quad (1c)$$

$$\frac{dD_p}{dt} = k_p D_{II}^l - (k_D + \mu)D_p \quad (1d)$$

$$\frac{dD_c^*}{dt} = k_1 R_I D_{II}^s - (k_{-1} + k_2 + k_3 M + \mu)D_c^* + k_{-2}D_c + k_{-3}D_M \quad (1e)$$

$$\frac{dD_c}{dt} = k_2 D_c^* - (k_{-2} + k_{-c} + \mu)D_c + k_4 D_M \quad (1f)$$

$$\frac{dD_M}{dt} = k_3 M D_c^* - (k_{-3} + k_4 + \mu)D_M \quad (1g)$$

$$\frac{dR_I}{dt} = k_I D - (k_1 D_{II}^s + \varepsilon_I + \mu) R_I + k_{-1} D_c^* \quad (1h)$$

$$\frac{dR_{II}}{dt} = k_{-I} D_{II}^I - (\varepsilon_{II} + \mu) R_{II} + k_D D_p \quad (1i)$$

$$\frac{dM}{dt} = k_M D - (k_3 D_c^* + \varepsilon_M + \mu) M + (k_{-3} + k_4) D_M \quad (1j)$$

The first equation (eqn (1a)) describes the change in free plasmid DNA concentration. Plasmid DNA is replicated at rate k_D from a plasmid DNA with an attached RNA II acting as replication primer (D_p). The factor 2 in equation (1a) appears because each round of replication doubles the number of plasmid DNA molecules in the cell. Formation of D_{II}^s (plasmid DNA with a short pre-primer RNA II attached) occurs at rate k_{II} per plasmid and decreases the concentration of free plasmid D. Dissociation of RNA II from D_{II}^I, at rate k_{-I} per molecule, regenerates a free plasmid. Plasmids with a stable RNA I–RNA II complex attached (D_c) lose the RNA I–RNA II complex at rate k_{-c} per complex and return to the free plasmid state D.

The other equations are similarly interpreted. The term proportional to μ appearing in all equations accounts for the decrease in concentration of cellular components due to the increase of cytoplasmic volume in cells growing exponentially with rate constant μ (μ = (ln 2)/doubling time). To see this, let $N(t)$ be the number of molecules of some intracellular component and let $V(t)$ be the total cytoplasmic volume of the cell culture, both at time t.

Table 1

Parameters used in the numerical integration of equations (1a) to (1j)

Rate constant	Reaction	Estimate (min^{-1})[a]		Source
		Wild-type	*inc*1*inc*2	
k_1	D_c formation	$1{\cdot}50 \times 10^8$ M^{-1}	$1{\cdot}25 \times 10^8$ M^{-1}	b
k_{-1}	D_c^* break-up	48	64	b
k_2	D_c formation from D_c^*	44	11	b
k_{-2}	D_c break-up	0·085	0·17	b
k_3	D_M formation	$1{\cdot}70 \times 10^8$ M^{-1}	$3{\cdot}15 \times 10^7$ M^{-1}	b
k_{-3}	D_M break-up	0·17	0·17	b
k_4	D_c formation from D_M	34	34	b
k_I	Synthesis of RNA I	6	6	c
k_{II}	Synthesis of RNA II	0·25	0·25	c
k_M	Synthesis of Rom protein	4	4	c
k_t	Elongation of susceptible RNA II transcripts	12	12	d
k_{-I}	Release of RNA II transcripts	4·3	4·3	e
k_p	Primer formation	4·3	4·3	e
k_D	Plasmid replication	5	5	f
k_{-c}	Release of stable complex	17	17	g
ε_I	RNA I degradation	0·35	0·35	Half-life of 2 min[h]
ε_{II}	RNA II degradation	0·35	0·35	Half-life of 2 min[h]
ε_M	Rom protein turnover	0·14	0·14	Half-life of 5 min
μ	Cell growth	0·0087	0·0087	Doubling time of 80 min

[a] The parameters were taken from the literature or estimated as indicated in the last column.

[b] Parameters k_1, k_{-1}, k_2, k_{-2}, k_3, k_{-3}, and k_4 were previously estimated from *in vitro* experiments at 25 °C (Tomizawa & Som, 1984; Perelson & Brendel, 1989). The *in vitro* estimates were multiplied by a factor of 1·7 to account for the increased reaction rate at 37 °C (Brenner & Tomizawa, 1991). The parameter estimates by Perelson & Brendel were made assuming that the unstable RNA I–RNA II complex was in quasi-steady-state. Although one of the sufficient conditions given in Perelson & Brendel for the existence of a quasi-steady-state, $k_1 R_I \ll k_{-1} + k_2$, is violated at the high RNA I concentration found *in vivo*, Segel & Perelson (1992) derived a less restrictive sufficient condition that is satisfied for the parameter values given in the Table.

[c] The synthesis rates k_I, k_{II}, and k_M were obtained from a search of the parameter space, with all other reaction rates kept fixed at the values indicated in the Table. The search was conducted for parameter combinations for which equations (1a) to (1j) give steady-state values in the range of the experimentally determined concentrations (per average cell volume: 10 to 30 plasmid copies; 200 to 600 RNA I molecules; 1 to 5 RNA II molecules; 200 to 2000 Rom protein monomers; Brenner & Tomizawa, 1991). The values given in the Table are representative. For k_I values somewhat smaller or larger (3 to 7·5 min^{-1}), a corresponding k_{II} could be obtained by preserving the ratio k_I/k_{II} of about 20 to 30.

[d] RNA II transcripts are susceptible to inhibition of primer formation by RNA I while their length is between 110 and 360 nucleotides (Tomizawa, 1986). For an RNA polymerase transcription rate of about 50 residues/s (von Hippel *et al.*, 1984), a transcript should be reactive for an average lifetime of 5 s. We thus assume it disappears with rate constant $k_t = 1/5\ s^{-1}$.

[e] The average length of RNA II is 600 to 800 bases (Tomizawa *et al.*, 1981), and thus the average lifetime of D_{II}^I, $1/(k_{-I} + k_p)$, is about 7 s. About half of the transcripts become primers and the other half are elongated and released (Itoh & Tomizawa, 1980; Tomizawa & Itoh, 1981; Masukata & Tomizawa, 1984, 1986). Thus we take $k_p = k_{-I} = 4{\cdot}3\ min^{-1}$.

[f] The rate of plasmid replication (k_D) is obtained as the ratio of the elongation rate of DNA polymerase (about 500 bases/s) (Alberts *et al.*, 1983, p. 221) and the length of the plasmid (about 6000 base-pairs), giving $k_D \simeq 5\ min^{-1}$.

[g] The rate constant k_{-c} for release of stable complex from DNA is not known. Because the formation of a stable RNA I–RNA II complex prevents the RNA II from acting as a primer, we assume it destabilizes the interactions of RNA II with the plasmid DNA, and leads to its release. We therefore have chosen k_{-c} to be large. The exact value within 2 orders of magnitude is immaterial to the behavior of the model.

[h] According to Ataai & Shuler (1986) and Brenner & Tomizawa (1991).

Then the concentration $C(t) = N(t)/V(t)$ changes according to:

$$\frac{dC}{dt} = \frac{1}{V}\frac{dN}{dt} - \frac{1}{V}\frac{dV}{dt}C. \quad (2)$$

The first term corresponds to changes in concentration due to molecular reactions while the second term corresponds to concentration decrease due to dilution. For exponential volume increase $V(t) = V_0 \exp(\mu t)$, and the dilution term is simply $-\mu C$.

Although the set of equations (1a) to (1j) describes a complex reaction scheme, it must still be considered only an approximation to the intracellular biochemistry. Additional unstable intermediates exist in the various reactions among RNA I, RNA II, and Rom protein (Tomizawa, 1990*a*,*b*). As our previous model (Perelson & Brendel, 1989) adequately summarizes the *in vitro* kinetics of RNA I binding to RNA II in the presence and absence of Rom protein without taking account of these other intermediates, we have not included them in the above *in vivo* model either. We have modeled RNA I as a single species, ignoring the fact that during its degradation shorter forms of the molecule are produced, which may have residual RNA II binding activity (Tomizawa, 1984; Tomcsányi & Apirion, 1985; Tamm & Polisky, 1985). These shorter forms appear to be rapidly degraded (Lin-Chao & Cohen, 1991), so that it is unlikely that they would build up to concentrations that would interfere with functional RNA I.

Equations (1a) to (1j), due to the several nonlinear terms, are too complicated to allow an analytical solution, even for the algebraic problem of finding steady-state solutions. We have therefore computed solutions numerically using standard methods. Initial parameter estimates were obtained as explained in the footnotes to Table 1. Given these parameter estimates and insights obtained from our numerical analysis, we derive in the Appendix a simple formula (eqn (3) below) for the steady-state plasmid concentration. Using this formula, we discuss the dependence of the plasmid copy number on parameters and contrast our results with a formula derived by Brenner & Tomizawa (1991).

3. Model Predictions

Numerical integration shows that there is a single positive steady-state solution to equations (1a) to (1j). Thus in an exponentially growing cell culture, in the absence of selection, the average plasmid concentration should be constant. The steady-state values for plasmid, RNA I and II, and Rom protein corresponding to our default parameter estimates (Table 1) are shown in Table 2; the other variables attain comparatively negligible values. We consider both wild-type and a mutant plasmid, *inc*1*inc*2. The *inc*1*inc*2 mutant incorporates two C·G pair to A·T pair replacements in the loop regions of RNA I and RNA II, resulting in weaker hydrogen bonding at the initial stage of complex formation. This entails a lower estimate for k_1 (Table 1) with corresponding higher steady-state plasmid copy number (formula (3) below). Brenner & Tomizawa (1991) measured RNA I concentrations of about 1 μM, RNA II concentrations of about 7 nM, and Rom protein concentrations in the range 1 μM to 5 μM for wild-type plasmid. Our estimates for the synthesis rate constants, k_I, k_{II}, and k_M were adjusted to give steady-state values in the observed concentration range, as well as wild-type copy number around 10 to 30 plasmids per average cytoplasmic cell volume (calculated from Brenner & Tomizawa's data to be about $6{\cdot}25 \times 10^{-16}$ liter). Plasmid copy number was not directly measured by Brenner & Tomizawa. The values given in Table 2 are predicted for the specific growth conditions in their experiments, corresponding to a generation time of 80 minutes. For a generation time of 30 minutes ($\mu = 0{\cdot}023\ \text{min}^{-1}$), our model gives a steady-state value of 11 plasmids per cytoplasmic volume as above. As faster-growing cells are larger by a factor of 1·5 to 2 (Pierucci, 1978; Kubitschek, 1990), we would predict values of 15 to 20 plasmids per cell under such growth conditions. Rom protein in the model enhances inhibition of replication by a factor of 1·9 (wild-type) to 4·3 (*inc*1*inc*2), magnitudes that are consistent with experimentally determined values (Tomizawa & Som, 1984).

Plasmid concentration is dependent upon both the growth phase and the growth rate of the host

Table 2

Model predictions from numerical integration of equations (1a) to (1j) with parameters as listed in Table 1. Initial conditions in the numerical integrations were set as follows: $D(0) = 4{\cdot}0 \times 10^{-8}$ M; $R_I(0) = 10^{-6}$ M; $M(0) = 10^{-6}$ M; all other variables 0

	Wild-type		*inc*1*inc*2	
	rom$^+$	*rom*$^-$	*rom*$^+$	*rom*$^-$
Plasmid copy number[a]	28	52	47	204
RNA I (M)	$1{\cdot}2 \times 10^{-6}$	$2{\cdot}2 \times 10^{-6}$	$2{\cdot}0 \times 10^{-6}$	$8{\cdot}4 \times 10^{-6}$
RNA II (M)	$5{\cdot}5 \times 10^{-9}$	$1{\cdot}0 \times 10^{-8}$	$9{\cdot}4 \times 10^{-9}$	$4{\cdot}8 \times 10^{-8}$
Rom protein (M)	$2{\cdot}0 \times 10^{-6}$	0	$3{\cdot}3 \times 10^{-6}$	0

[a] Plasmid copy number is given per average cytoplasmic volume of a cell, taken to be $6{\cdot}25 \times 10^{-16}$ liter (calculated from Brenner & Tomizawa, 1991).

bacteria (Seo & Bailey, 1985; Lin-Chao & Bremer, 1986; Fitzwater *et al.*, 1988). For example, Seo & Bailey (1985) find that plasmid concentration increases when the specific growth rate is reduced by changing the medium composition. A simple explanation for this phenomenon is indicated by the influence of changes in the growth parameter μ on plasmid concentration. Our model predicts a linear increase of steady-state plasmid concentration as a function of the doubling time over a wide range of parameter values. This prediction can be understood from equations (1a) to (1j) in the following way. Let $D_T = D + D_{II}^s + D_{II}^l + D_p + D_c^* + D_c + D_M$ be the total plasmid concentration. In the Appendix we show that the steady-state plasmid concentration $\bar{D}_T$ obeys the equation:

$$\bar{D}_T \simeq \frac{p k_1 \varepsilon_I \rho}{k_1 \left(\frac{k_I}{k_{II}} - \rho \right) \mu}, \tag{3}$$

where:

$$p = \frac{k_p}{k_{-1} + k_p}$$

is the fraction of long RNA II transcripts (not complexed with RNA I) that become primer and:

$$\rho = 1 + \frac{k_{-1}}{k_2 + k_3 M}$$

gives the dependence of the plasmid copy number on the Rom protein concentration M. In deriving equation (3), we have assumed $k_I > \rho k_{II}$ and $p k_{II} \gg \mu$. For our parameter estimates (Table 1), $p = 0{\cdot}5$, $\rho = 1{\cdot}1$ (wild-type), $\rho = 1{\cdot}6$ (*inc*1*inc*2), $k_I = 6$, and $k_{II} = 0{\cdot}25$. Thus, for a generation time of 30 minutes or longer (corresponding to $\mu < 0{\cdot}023$) the two assumptions are satisfied and $\bar{D}_T$ is seen to increase linearly with $1/\mu$ as predicted by our numerical solutions.

A linear dependence of plasmid concentration with respect to generation time is consistent with measurements by Lin-Chao & Bremer (1986) of plasmid pBR322 concentration as a function of host growth rate, where plasmid concentration was measured as plasmid per absorbance unit; see also Keasling & Palsson (1989*a*). The increase in plasmid concentration in cells with a longer doubling time is a consequence of the slowing down of cell growth dependent dilution, which leads to higher intracellular plasmid concentration. Even though the concentrations of both RNA I and Rom protein also increase, their build up is not sufficient to prevent the accumulation of replication primed plasmid DNA, D_p. In fact, the model predicts that for $\mu = 0$, i.e. no cell growth, runaway replication occurs. *In vivo*, high plasmid concentration will affect the availability of polymerase and other resources (not taken into account in our model) and prevent unbounded plasmid replication. Stopping cell growth by the addition of chloramphenicol results in an about 125-fold amplification of ColE1 plasmid copy number (Clewell, 1972).

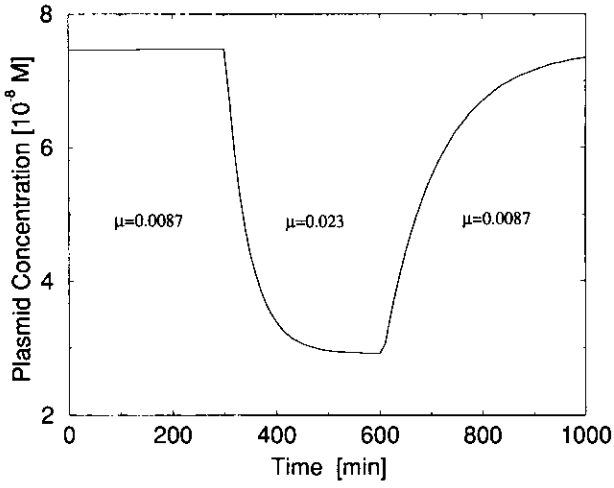

Figure 2. Dependence of plasmid concentration on the growth rate μ. The graph mimics a nutritional shift experiment in which growth conditions are changed at 300 min (from a generation time of 80 min ($\mu = 0{\cdot}0087$) to a generation time of 30 min ($\mu = 0{\cdot}023$)) and at 600 min (back to the initial conditions). The graph was obtained by numerical integration of equations (1a–j), with the initial plasmid concentration set arbitrarily close to the steady state concentration.

Figure 2 shows the prediction for a typical nutritional upshift experiment, simulated as a switch from a generation time of 80 minutes ($\mu = 0{\cdot}0087$) to a generation time of 30 minutes ($\mu = 0{\cdot}023$) and back. Note that $\bar{D}_T$ is given in units of molarity rather than copy number per average cell, because the average cell volume changes with the growth rate, and we only have available the Brenner & Tomizawa (1991) measurement of cell volume at the longer generation time. The predicted results for the upshift experiment are qualitatively similar to those found by Bremer & Lin-Chao (1986). However, Bremer & Lin-Chao (1986) found that there was a lag after the nutritional shift before molecular production proceeded at the new rate. This delay is not reproduced with our model because we have assumed the synthesis rate constants k_I, k_{II}, etc. to be independent of the growth rate μ, while in reality these parameters appear to be dependent on the growth rate (Lin-Chao & Bremer, 1986). Our assumption is a practical simplification, and one that can be lifted if enough data become available. However, this practical simplification may not be a great limitation. Bremer & Lin-Chao (1986) noted that whatever the parameter dependences on growth rate they seem to compensate one another such that the prediction of plasmid concentrations based on constant parameters remains reliable. This fact may derive from the dependence of the steady-state plasmid concentration on the RNA synthesis rates *via* the ratio k_I/k_{II} (see eqn (3) and Fig. 5(a), discussed in the next section), which would remain constant if k_I and k_{II} depended on μ in the same way. In experiments by Gustafsson & Nordström (1980), the rate of plasmid R1 replication was

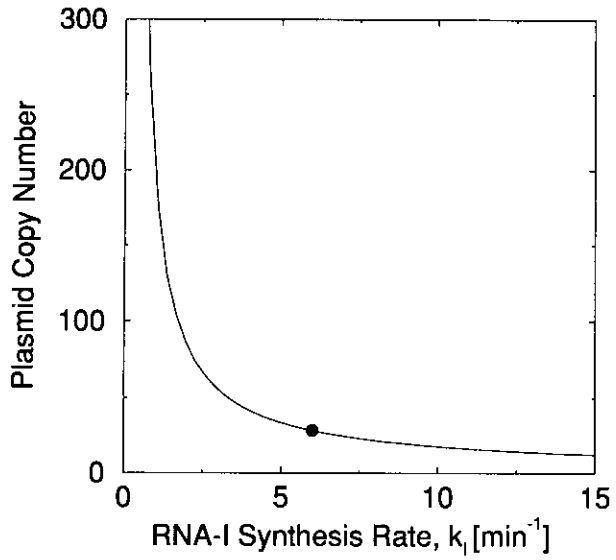

Figure 3. Dependence of the steady-state wild-type plasmid copy number on the synthesis rate k_I of RNA I. If no or little functional RNA I is synthesized ($k_I < 0{\cdot}5\ \mathrm{min}^{-1}$) the model predicts runaway replication of plasmid. At higher synthesis rates of RNA I plasmid copy number is maintained at a stable steady-state. Our default value (Table 1) is $k_I = 6\ \mathrm{min}^{-1}$ (indicated by ●), with predicted copy number 28 plasmids per average cell volume.

shown to adjust immediately under experimental conditions that shift the growth rate without changing the composition of the cells (i.e. without changing the metabolic rates).

4. Mechanism of Replication Inhibition

To study the nature of the inhibitory mechanism, we have simulated various mutations affecting any single parameter of the model. As RNA I plays the role of a replication inhibitor, we were particularly interested in parameters controlling the *in vivo* RNA I concentration. Figure 3 shows the influence of the RNA I synthesis rate constant k_I on steady-state copy number of wild-type plasmid. If little or no RNA I is synthesized, $k_I \lesssim 1{\cdot}25\ \mathrm{min}^{-1}$, the copy number increases to very high levels. Eventually, as k_I becomes as low as ρk_{II} ($\simeq 0{\cdot}28$ for wild-type parameters), copy number control is lost and the model predicts runaway replication (eqn (3)). At higher RNA I synthesis rates plasmid copy number is maintained at a stable steady-state level.

Copy number is similarly sensitive to the RNA I decay rate constant (ε_I), increasing linearly with this parameter (Fig. 4(a), eqn (3)). In Figure 4(b) we have plotted the steady-state number of RNA I molecules per plasmid *versus* ε_I. For $\varepsilon_I = 0{\cdot}35\ \mathrm{min}^{-1}$ (as given in Table 1) this ratio is about 16, and in general, as shown in the Appendix, the ratio is approximately k_I/ε_I. Fitzwater *et al.* (1988) calculate the ratio to be about 10 to 20 for the ColE1-derived mutant plasmids pMM7 and pNOP42 in exponentially growing cells (the quoted ratio obtains after

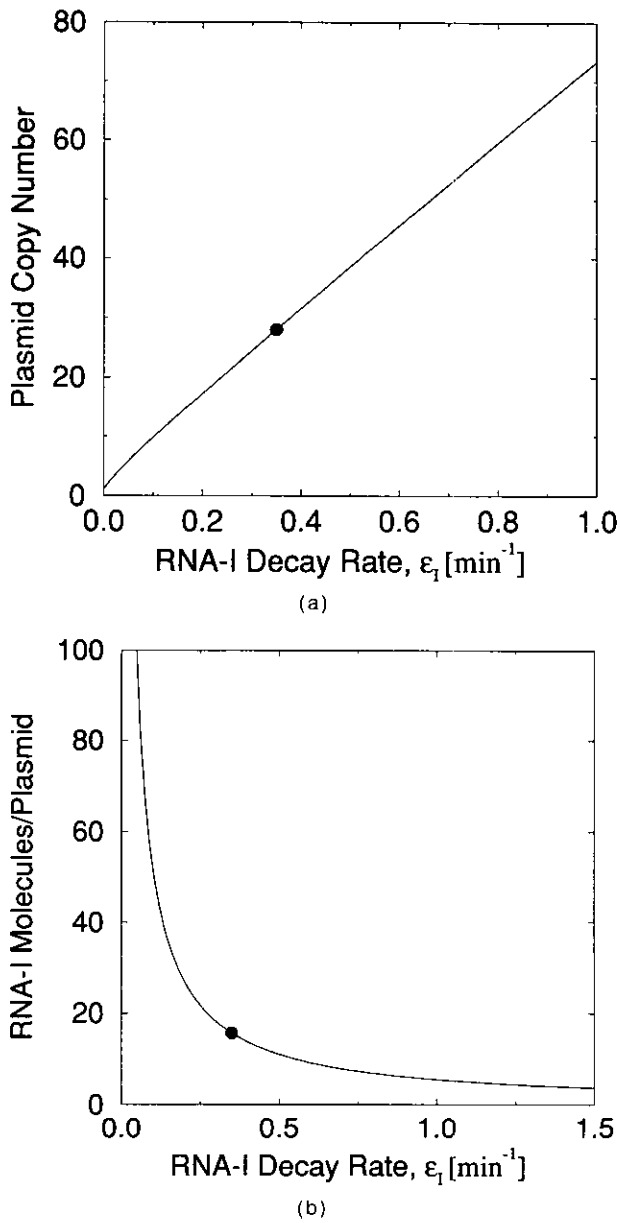

Figure 4. (a) Dependence of the steady-state wild-type plasmid copy number on the decay rate ε_I of RNA I. (b) Steady-state number of RNA I molecules per plasmid as a function of the RNA I decay rate. Our default value (Table 1) is $\varepsilon_I = 0{\cdot}35\ \mathrm{min}^{-1}$ (indicated by ●), with predicted copy number 28 plasmids per average cell volume and RNA I/plasmid ratio 16.

subtraction of inactive RNA I_{-5} from the measured RNA I fraction). Lin-Chao & Bremer (1987) estimate ε_I as $1{\cdot}25\ \mathrm{min}^{-1}$, giving a ratio of 4·5 according to our model (Fig. 6(b)) compared to their observed values between 1·0 and 1·35, depending on the growth medium.

Plasmid copy number is also sensitive to the parameters determining primer formation and processing (k_{II}, k_t, k_{-t}, k_p). Figure 5 shows the dependence on the rate of transcription. If the RNA I and RNA II promoters are coregulated (as we would expect if both promoters were under

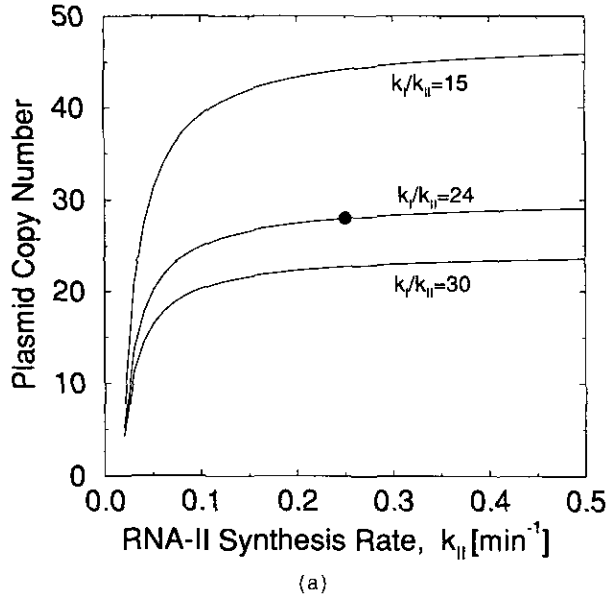

Figure 5. (a) Copy number as a function of the overall transcription rate. Numbers are plotted *versus* the RNA II synthesis rate (k_{II}) with the ratio of the RNA I to RNA II synthesis rates held constant at the indicated values. (b) Copy number as a function of the RNA II synthesis rate; unlike panel (a), here for each graph the RNA I synthesis rate is kept constant at the indicated value. Our default values (Table 1) are $k_I = 6$ min and $k_{II} = 0{\cdot}25$ min^{-1} (indicated by ●), with a predicted copy number of 28 plasmids per average cell volume.

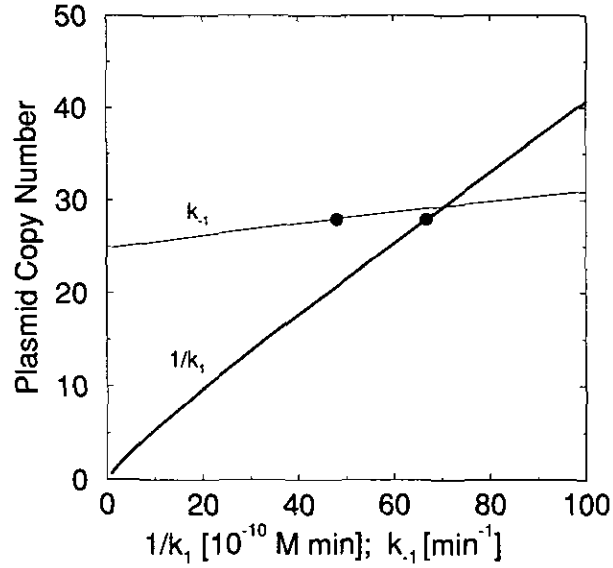

Figure 6. Dependence of the steady-state wild-type plasmid copy number on the rates of unstable RNA I–RNA II complex formation (k_1), indicated by the dark line, and dissociation (k_{-1}), indicated by the light line. Our default values (Table 1) are $k_1 = 1{\cdot}50 \times 10^8$ M^{-1} min^{-1} and $k_{-1} = 48$ min^{-1} (indicated by ●), with a predicted copy number of 28 plasmids per average cell volume.

general metabolic control) then the ratio of transcription rates, k_I/k_{II}, should remain constant. Figure 5(a) shows the dependence of the copy number on k_{II}, with the ratio k_I/k_{II} held at various constant values. For high values of the overall transcription rate, the copy number reaches an asymptote that depends only on the k_I/k_{II} ratio but not on the magnitude of the overall transcription rate. This is as predicted from equation (3), which is valid for $k_{II} \gg \mu/p$. For the parameters of Table 1, $\mu/p =$ 0·017, and thus the asymptotic value for a given k_I/k_{II} ratio is virtually attained for $k_{II} = 0{\cdot}1$ in agreement with the plots in Figure 5(a). Figure 5(b) shows the effect of specific regulation of only the RNA II promoter. Plasmid copy number is seen to increase linearly with the RNA II synthesis rate constant (see eqn (3) for $k_I \gg \rho k_{II}$). The effects displayed in Figure 5(a) have been confirmed qualitatively under conditions that reduce the k_I/k_{II} ratio (Polaczek & Cieśla, 1984) and with plasmid constructs in which the primer promoter was replaced by a stronger promoter (Panayotatos, 1984); however, quantitative verification is outstanding.

Mutations that completely debilitate or prevent synthesis of Rom protein result in threefold (wild-type) to 6·7-fold (*inc*1*inc*2) increase in copy number (Tomizawa & Som, 1984). The factor ρ in equation (3) predicts a similar increase, since $\rho = 1{\cdot}1$ at the calculated *in vivo* concentration of Rom protein (Table 2) and $\rho = 2{\cdot}1$ for the *rom*$^-$ wild-type mutant, whereas ρ changes from 1·6 to 6·8 for the *inc*1*inc*2 mutant when Rom protein is absent. Changes in the kinetic parameters characterizing Rom protein action lead to smaller changes in copy number. For example, we have assumed that Rom protein has a half-life of five minutes ($\varepsilon_M =$ 0·14 min^{-1}; Table 1). Reducing the half-life to one minute ($\varepsilon_M = 0{\cdot}28$ min^{-1}), which decreases the Rom protein concentration by a factor of 2, only increases the average copy number from 28 to 30 plasmids per cell. Changing k_M, the Rom protein synthesis rate constant, from its value of 4 min^{-1} in

Table 1 over the range 0·1 min^{-1} to 10 min^{-1} yields average copy numbers of 46 to 26. There is a similar insensitivity to changes in the kinetic parameters k_3, k_{-3}, and k_4, which determine the interaction of Rom protein with the unstable RNA I–RNA II complex.

Figure 6 shows the variation in copy number with changes in the values of k_1 and k_{-1}, the rate constants for formation and dissociation of the unstable RNA I–RNA II complex. Whereas changes in k_1 have dramatic consequences, changes in k_{-1} over several orders of magnitude have much less influence on copy number. The critical dependence on k_1 is in agreement with equation (3), which shows that the steady-state plasmid concentration is proportional to $1/k_1$. This is the dependence shown in Figure 6. The other rate constants describing the interactions among RNA I, RNA II, and Rom protein (k_{-1}, k_2, k_{-2}, k_3, k_{-3} and k_4) affect the plasmid concentration indirectly through the value of R_I. This is not to say that they have no effect. In fact, we have previously shown (Perelson & Brendel, 1989) that the maximum enhancement in the rate of RNA I–RNA II binding due to Rom protein is given by $1+k_{-1}/k_2$, a factor that also approximates the increase in copy number in *rom*$^-$ mutants (see below).

5. Discussion

ColE1 is a multicopy plasmid that is stably maintained in bacterial cells even in the absence of selection. The proposed mechanism of ColE1 copy number control relies upon inhibition of replication initiation through plasmid-encoded RNA I, which is complementary to the 5′-end of RNA II, a transcript that serves as a primer for DNA synthesis. Complex formation between the two complementary RNAs interferes with the capability of RNA II to serve as primer and thus inhibits initiation of plasmid DNA replication.

The critical reaction in this inhibition mechanism appears to be the formation of a transient complex of RNA I with RNA II. This unstable complex, formed by weak interactions between complementary loops in the secondary structures of the two RNAs, precedes stable complex formation due to hydridization. Rom, a plasmid-specified protein, enhances the rate of stable complex formation by interacting with the unstable complex and facilitating its conversion into stable complex (Tomizawa, 1990*b*; Eguchi & Tomizawa, 1990). The binding reaction, in the presence and absence of Rom protein, has been studied extensively *in vitro* (Tomizawa, 1984, 1985; Tomizawa & Som, 1984; Dooley & Polisky, 1987). We previously presented a kinetic model of the reaction that accounts for the observed data and allows the estimation of the reaction parameters (Perelson & Brendel, 1989). Here we have shown that the RNA I–RNA II–Rom protein reaction model extends to a functional copy number control mechanism under putative *in vivo* conditions.

As the replication inhibitor, RNA I, is plasmid-encoded and constitutively expressed, the basic principle of the inhibition mechanism relies on the essentially second-order reaction between the inhibitor and its target. If the plasmid copy number in a particular cell is high, the inhibitor concentration will adjust at a correspondingly high level, resulting in a high probability of the inhibitory reaction. On the other hand, a low plasmid copy number induces a low inhibitor concentration, with associated low probability of inhibition. In either case, the adjustment is in the direction of the normal, steady-state plasmid copy number. Our numerical solutions to equations (1a) to (1j) confirm such behavior for the ColE1 system. Thus ColE1 replication control involves a negative-feedback loop and is in general agreement with the theoretically proposed inhibitor dilution model (Pritchard *et al.*, 1969; Pritchard, 1984).

While a number of models for ColE1 plasmid copy number control have been proposed that incorporate RNA I as a replication inhibitor (Bremer & Lin-Chao, 1986; Ataai & Shuler, 1986; Keasling & Palsson, 1989*a*,*b*; Bremer & Tomizawa, 1991), none of these previous models is comprehensive in the sense of including all of the pertinent RNA I–RNA II–Rom protein interactions, considering synthesis and degradation of components as well as dilution effects due to cell growth, and using independently obtained estimates of kinetic parameters. Here we have developed such a comprehensive model and have used the model to make a number of testable predictions. Parameters were fitted to match plasmid copy number and intracellular concentrations of RNA I, RNA II and Rom protein with the values measured by Brenner & Tomizawa (1991) for wild-type plasmid. The validity of the model and the parameter estimates could be tested by measurements for the *inc*1*inc*2 or other mutants. It is interesting that our model predicts that plasmid, RNA I, RNA II, and Rom protein all have higher intracellular concentrations for the *inc*1*inc*2 mutant than wild-type (Table 2). The model also serves to predict parameter sensitivity and the effects of mutations in any of the steps involved in the copy number control mechanism. Some of the predictions of our model are novel and differ from those made by Brenner & Tomizawa (1991) on the basis of steady-state calculations.

Our model predicts that the steady-state plasmid concentration increases linearly with the cell doubling time (which is the inverse of the growth or dilution rate). Furthermore, if cell growth is halted (as is customary in plasmid amplification by chloramphenicol treatment), the model (with $k_M = 0$ and $\mu = 0$) predicts runaway replication until other host factors limit plasmid duplication. Nutritional up- and downshift experiments may thus be an easy way to check the quantitative validity of the model: transfer from minimal to rich medium will decrease the cell doubling time and result in a decreased plasmid concentration, whereas transfer from rich to minimal medium will prolong the cell doubling

time and result in an increased plasmid concentration. Some observations in the literature seem to support these predictions (Seo & Bailey, 1985; Lin-Chao & Bremer, 1986; Bremer & Lin-Chao, 1986; Fitzwater *et al.*, 1988), but more precise measurements at different growth rates comparable to the Brenner & Tomizawa (1991) data would be helpful.

The critical parameter in RNA I-mediated inhibition of replication from our analysis appears to be k_1, the rate constant for unstable complex formation of RNA I with RNA II (see Fig. 6). This result is consistent with the effect of mutations in the loops of the RNAs, which are predicted to destabilize the loop–loop interactions and show increased copy numbers (as in the case of *inc*1*inc*2, Table 2; Tomizawa, 1985).

Rom protein, which enhances stable RNA I–RNA II complex formation, leads to a decrease in copy number both *in vivo* and in our model. Simulating *rom*$^-$ mutations by eliminating Rom protein from our model leads to a twofold (wild-type) or more than fourfold (*inc*1*inc*2) increase in plasmid copy number (Table 2). The magnitude of the copy number increase corresponds to the magnitude of the enhancement in the rate of stable RNA I–RNA II complex formation *in vitro* (Tomizawa & Som, 1984), further reinforcing the notion that stable RNA I–RNA II complex formation is the essential factor in copy number control. Although there are quantitative differences in copy number in the absence of Rom protein, we do not find any qualitative differences in the copy number control mechanism. It remains unclear whether there might be any evolutionary advantage to the restriction of copy number *via* Rom protein (as opposed to restriction of copy number *via* a higher RNA I synthesis rate, for example). A possibility is that Rom protein may have another function not included in our present model. Conceivably Rom protein may bind to singular RNA I molecules and protect them from excessive degradation in certain host environments. This would be a vital function, since excessive degradation of RNA I leads to loss of plasmid copy number control (see Fig. 4(a)).

While excessive degradation of RNA I might cause runaway plasmid replication, an overly stable RNA I molecule (ε_I small; see Fig. 4(a)) would cause the accumulation of replication inhibitor and would restrict plasmid replication beyond the level required for stable plasmid maintenance. Thus, the proper stability of the inhibitor is a vital ingredient of the replication control mechanism (as already proposed by Pritchard *et al.*, 1969). ColE1 RNA I degradation *in vivo* occurs largely *via* an RNase E-mediated pathway at a rate that is independent of the rate of cell growth; mutations affecting this pathway alter the specific plasmid copy number (Lin-Chao & Cohen, 1991).

The inhibitor RNA I interacts stoichiometrically with RNA II. Thus, one might expect the RNA I and RNA II promoters to be coregulated *in vivo*. We show (see Fig. 5(a)) that if this is the case, then for high values of the transcription rate, copy number will depend only on the ratio k_I/k_{II} of the RNA I to RNA II transcription rates but not on the individual values of k_I and k_{II}. However, at low transcription rates, copy number falls due to insufficient primer synthesis. Mimicking experiments in which the RNA II promoter is replaced by an inducible promoter so that k_{II} can be controlled independently of k_I (Panayotatos, 1984; Polaczek & Cieśla, 1984), we find that copy number can be varied over two orders of magnitude (see Fig. 5(b)).

In the Appendix we derive equation (3) for the steady-state plasmid concentration. Inserting our estimates $p = 0{\cdot}5$ for the fraction of uncomplexed RNA II that become primer and $k_l = 12$ for the rate of elongation of RNA II molecules that are susceptible to inhibition of primer formation by RNA I, we obtain:

$$\bar{D}_T \simeq 6\,\frac{\varepsilon_I \rho}{k_1\left(\frac{k_I}{k_{II}} - \rho\right)\mu}, \qquad (4)$$

$$\rho = 1 + \frac{k_{-1}}{k_2 + k_3 M}.$$

For a *rom*$^-$ mutant:

$$\left(M = 0, \quad \rho = \rho_{\max} = 1 + \frac{k_{-1}}{k_2}\right),$$

$\bar{D}_T$ *is increased by a factor of:*

$$\frac{\rho_{\max}}{\rho}\left(\frac{k_I}{k_{II}} - \rho\right)\Big/\left(\frac{k_I}{k_{II}} - \rho_{\max}\right)$$

compared to the steady-state plasmid concentration in the presence of Rom protein. As $\rho = 1$ at very high Rom protein concentrations, the maximal increase in plasmid concentration due to a *rom*$^{-1}$ mutation is seen to be:

$$\left(1 + \frac{k_{-1}}{k_2}\right)\left(\frac{k_I}{k_{II}} - 1\right)\Big/\left(\frac{k_I}{k_{II}} - 1 - \frac{k_{-1}}{k_2}\right).$$

Here the first factor:

$$1 + \frac{k_{-1}}{k_2},$$

corresponds exactly to the relative enhancement of complex formation in the presence of Rom protein for the *in vitro* reaction of RNA I and RNA II (Perelson & Brendel, 1989). Thus the *in vivo* effect of Rom protein to decrease copy number is somewhat larger than its *in vitro* effect of enhancing complex formation, a correlation that was experimentally established by Tomizawa & Som (1984). It is noteworthy that for our parameter estimates, $\rho = 1{\cdot}1$ (wild-type) or $\rho = 1{\cdot}6$ (*inc*1*inc*2), ρ values are larger than the minimum value 1. This suggests that *in vivo* Rom protein occurs at concentrations not quite large enough to exert its maximal effect on copy number restriction. Experiments in which Rom protein is supplied *in trans* from an inducible promoter might clarify these quantitative predictions.

Brenner & Tomizawa (1991) derive an equation similar to (4) based on a number of greatly simplifying steady-state assumptions. In our notation and units their equation reads:

$$\bar{D}_T = 12 \frac{\varepsilon_I}{k_1 k_I} \ln \frac{k_{II}}{\mu}.$$

Here the value 12 replaces 6 in equation (4), because Brenner & Tomizawa ignore the fraction of uncomplexed RNA II transcripts failing to hybridize to the region around the origin of replication, effectively setting $p = 1$. In the parameter range $k_I/k_{II} \gg \rho$, $\rho \simeq 1$, equation (4) reduces to:

$$\bar{D}_T \simeq 6 \frac{\varepsilon_I}{k_1 k_I} \frac{k_{II}}{\mu}. \tag{5}$$

Aside from the different assignments for p, our model agrees with Brenner & Tomizawa's formula in predicting a linear dependence of plasmid copy number on ε_I (see also Fig. 4(a)), $1/k_1$ (Fig. 5(b)), and $1/k_I$ (Fig. (3)). However, the Brenner & Tomizawa formula and equation (5) differ significantly in their dependence on k_{II}/μ, since equation (5) also indicates a linear dependence on k_{II} (Fig. 6) and $1/\mu$, and not a logarithmic dependence. Thus, whereas Brenner & Tomizawa argue that copy number is relatively insensitive to the RNA II synthesis rate, we predict a strong sensitivity in the case that the RNA II promoter is regulated independently of the RNA I promoter. For example, according to equation (5) a threefold increase in copy number is caused by a threefold increase in the synthesis rate constant k_{II}, whereas the Brenner & Tomizawa formula requires a $(k_{II}/\mu)^2$-fold increase in k_{II} to produce the same effect on copy number. For our parameter estimates (Table 1), $(k_{II}/\mu)^2 = 826$. So, the difference in prediction is dramatic. It should be noted that the magnitude of the difference depends upon the exact value of k_{II}. Brenner & Tomizawa (1991) estimate k_{II} analogously to k_I as $\varepsilon_{II}(\bar{R}_{II}/\bar{D}_T)$, which gives smaller values than our estimate. However, their argument does not take into account the different pathways of RNA II turnover *via* complex with RNA I, primer, or nascent transcript, and involves a concomitant estimate for the k_I/k_{II} ratio as $\bar{R}_I/\bar{R}_{II} \simeq 400/3$, a ratio that seems to be excessively high in view of other authors' estimates in the range of 3 to 6·5 (Lin-Chao & Bremer, 1986, 1987; Ataai & Shuler, 1986). Further quantitative experiments are necessary to settle these differences.

Appendix

We derive here equation (3) in the text. Steady-state concentrations will be denoted by overbars. From equation (1d) of the text:

$$\bar{D}_p = \frac{k_p}{k_D + \mu} \bar{D}^l_{II} \simeq \frac{k_p}{k_D} \bar{D}^l_{II} \tag{A1}$$

(since $k_D \gg \mu$, see Table 1). Similarly, from equation (1c):

$$\bar{D}^l_{II} \simeq \frac{k_l}{k_{-1} + k_p} \bar{D}^s_{II}, \tag{A2}$$

from equation (1b):

$$\bar{D}^s_{II} \simeq \frac{k_{II}\bar{D} + k_{-1}\bar{D}^*_c}{k_l + k_1 \bar{R}_I}, \tag{A3}$$

and from equation (1e), using the result from our numerical analysis that $k_{-2}\bar{D}_c + k_{-3}\bar{D}_M \ll k_1 \bar{R}_I \bar{D}^s_{II}$:

$$\bar{D}^*_c \simeq \frac{k_1 \bar{R}_I \bar{D}^s_{II}}{k_{-1} + k_2 + k_3 \bar{M}} \tag{A4}$$

By adding equations (1a) through (1g) of the text one finds:

$$\frac{dD_T}{dt} = k_D D_p - \mu D_T, \tag{A5}$$

where D_T is the total plasmid concentration. Therefore, at steady-state:

$$\bar{D}_T = \frac{k_D}{\mu} \bar{D}_p, \tag{A6}$$

and after inserting successively the above expressions for $\bar{D}_p$ and $\bar{D}^l_{II}$:

$$\bar{D}_T \simeq \frac{p k_l}{\mu} \bar{D}^s_{II}. \tag{A7}$$

Here $p = k_p/(k_{-1} + k_p)$ is the fraction of long RNA II transcripts (not complexed with RNA I) that become primer. By equation (1h):

$$\bar{R}_I = \frac{k_I \bar{D} + k_{-1}\bar{D}^*_c}{k_1 \bar{D}^s_{II} + \varepsilon_I + \mu} \simeq \frac{k_I}{k_1 \bar{D}^s_{II} + \varepsilon_I} \bar{D}, \tag{A8}$$

using the result from our numerical analysis that $k_I \bar{D} \gg k_{-1}\bar{D}^*_c$.

Also from our numerical analysis, we know that almost all plasmid DNA is in free form, D. Therefore we may replace $\bar{D}$ by $\bar{D}_T$ in equations (A3) and (A8). Insertion of equations (A4) and (A7) into equation (A3) then gives an equation for $\bar{R}_I$ with solution:

$$\bar{R}_I = \frac{k_l}{k_1}\left(\frac{p k_{II}}{\mu} - 1\right)\rho, \tag{A9}$$

where $\rho = 1 + k_{-1}/(k_2 + k_3\bar{M})$. Insertion of equations (A7) and (A9) into equation (A8) gives an equation for $\bar{D}_T$ with solution:

$$\bar{D}_T = \frac{\varepsilon_I \dfrac{k_l}{k_1}\left(\dfrac{p k_{II}}{\mu} - 1\right)\rho}{k_I - \left(k_{II} - \dfrac{\mu}{p}\right)\rho}. \tag{A10}$$

In a parameter range for which $p k_{II} \gg \mu$ the above expressions simplify to:

$$\bar{R}_I \simeq \frac{k_l p k_{II} \rho}{k_1 \mu} \tag{A11}$$

and:

$$\bar{D}_T \simeq \frac{pk_I\varepsilon_I\rho}{k_1\left(\frac{k_I}{k_{II}}-\rho\right)\mu}. \qquad \text{(A12)}$$

In the presence of excess Rom protein (M large, $\rho \simeq 1$):

$$\bar{D}_T \simeq \frac{pk_I\varepsilon_I}{k_1\left(\frac{k_I}{k_{II}}-1\right)\mu} \qquad \text{(A13)}$$

and:

$$\frac{\bar{R}_I}{\bar{D}_T} \simeq \frac{k_I-k_{II}}{\varepsilon_I} \simeq \frac{k_I}{\varepsilon_I}. \qquad \text{(A14)}$$

The agreement of equations (A11) and (A12) with our simulation results is close. Thus, for the wild-type parameters (see Table 1) equation (A11) predicts $\bar{R}_I = 1{\cdot}3\times10^{-6}$ M compared to the simulation result $1{\cdot}2\times10^{-6}$ M (see Table 2), and a steady-state copy number of 30 compared to 28 by simulation. The error is larger for the high-copy number mutant *inc1inc2-rom*$^-$ (eqn (A12) gives a steady-state copy number of 287, compared to the value 204 in Table 2). In this case, the assumptions $k_I\bar{D} \gg k_{-1}\bar{D}_c^*$ and $\bar{D} = \bar{D}_T$ of our derivation are not as good.

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