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The onset of macroscopically detectable amplification of template concentration for self-replicating RNA

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We calculate the strength of fluctuations in concentrations and rates for a self-replicating RNA system catalyzed by the Qβ-replicase at very low initial template concentration ($1-10^3$ strands/ml). The work is centered upon the derivation of the induction periods which must elapse in order for the rate-correlation size to become comparable to a kinetic barrier determined by the width of the probability distribution about the invariant portion of the concentration space. This surface is identified by a center manifold and corresponds to the subordination of relaxing kinetic modes to the overall growth of the total (free and complexed) template concentration. The results are compared with the experimental data for the onset of a macroscopically detectable amplification of template concentration and a satisfactory agreement is observed.

1. Introduction

The RNA self-replicating system, a template-instructed synthetic pathway actively catalyzed by the Qβ-replicase [1–3], is perhaps one of the few realistic examples of a kinetic cycle with overall autocatalysis. We shall concentrate here upon the onset of macroscopically detectable amplification in the concentration of template. In this respect, intrinsic fluctuations in the early stages, when the initial number of template strands is very low ($1-10^3$ macromolecules/ml), are crucial since their effect will be amplified in time leading to macroscopic consequences [3]. The aim of this work is to study a realistic experimental set-up and to determine precisely how the onset of a detectable synthetic rate arises. This point can only be clarified if we are able to determine the rate-fluctuation correlations during the very early stages of replication. In this respect, any extrapolation from results on near-equilibrium thermal fluctuations is of no avail [4–8]. The equilibrium law, variance = $O(Z^{1/2})$ (where Z is the number of template strands), is not valid in our case of interest as will be proven in this work. When the size of fluctuations reaches a certain threshold value B, after an induction period has elapsed, the exponential growth of template concentration becomes detectable. In order to interpret B properly, we must note that the probability function P is distributed along a locally attractive and locally invariant surface in concentration space: the center manifold (CM) [4–8]. This surface represents the statistical subordination of the rapidly relaxing kinetic modes to the overall growth of the total (free and complexed) template concentration. The Gaussian width of P depends on the position on the CM, i.e., on the CM coordinate given by the total

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template concentration. This fact enables us to calculate the initial critical template population which corresponds to a given induction period. Once the fluctuations attain the size of the Gaussian width, then the system restricts itself to the CM and thus to the statistical subordination of certain kinetic modes to the growth of the overall template concentration. At this stage, amplification of the template concentration becomes detectable. The rate-fluctuation correlations are scaled with functions of the control parameters of the system in order to yield an adequate competition between the deterministic drift towards the CM, provided by the replication kinetics [3], and the diffusion pressure given by the fluctuations [6,8]. From these scaling relations, we can derive the dependence of the induction periods on the substrate (nucleoside triphosphate, NTP) concentration. This parameter enters in the expression for the averaged elongation rate constant for the formation of the phosphodiester linkage, i.e., for the elementary step: $I_j \rightarrow I_{j+1}$, where I_j is an intermediate complex with a chain of j nucleotides of the original template sequence.

A remarkable feature which becomes apparent in this treatment is the extreme sensitivity of induction periods to the effective substrate dissociation equilibrium constant. This characteristic is illustrated in fig. 2 and can be taken advantage of by estimating the equilibrium constant which yields realistic values of the induction period for a given initial template concentration (cf. ref. 2). The range of initial template concentrations under examination is $1–10^3$ strands/ml. As suggested by fig. 1, the dramatic changes in size of the concentration fluctuations in this range imply that two different regimes can be clearly distinguished: (a) 1–10 strands/ml and (b) $10^2–10^3$ strands/ml. In the first, the width of P about the CM is sufficiently large to yield detectable induction periods, of the order of minutes, depending on the NTP concentration. The second regime, however, corresponds to a probability distribution which behaves like a Dirac-delta function peaking at the CM and the deterministic kinetics picture holds. It must be stressed, however, that even in this regime, the fluctuations are far greater than their near-equilibrium counterparts, the difference being three orders of magnitude for a typical concentration of 10^2 strands/ml. Significantly, such values were found in previous derivations making use of the stochastic CM theory as applied in a completely different realm: The strength of the actual random source in the transition to a convective roll pattern in a Rayleigh-Bénard cell was found to be 6000-times larger than the value obtained for the thermal fluctuations [4,5].

At this point some words of caution are in order: The critical template concentrations obtained on inspection of fig. 2 must be interpreted as effective concentrations and not as actual concentrations for the entire reacting mixture. This is so since fluctuations far from equilibrium are endowed with a local nature, in other words, they are confined to a virtual volume: The fluctuation correlation $\bar{C}$ does not scale with the inverse of the macroscopic volume, as is the case with near-equilibrium fluctuations; instead, its scaling is determined by the control rate parameters of the system (cf. section 4).

2. The reaction scheme

The template-instructed RNA replicator is an autocatalytic system where a complex formed by binding of the template to Qβ-replicase acts as the active catalyst [3]. The essential features of the replication mechanism are as follows:

(a) Binding of the enzyme to the active site of the RNA template, i.e., to the 3′-end of the strand. This process is extremely fast, with a rate constant estimated to be 10^7 s^{-1} [2,3].

(b) Elongation steps. The replica is produced by progressive incorporation of nucleotides in the $5' \rightarrow 3'$ direction. At the final stage of this process, the enzyme appears to bind to the 3′-end of the replica and to the (inactive) 5′-end of the template. The sequence is copied by means of the complementarity bond pairing of nucleotides in the Pauling sense [2,3]: A-U and G-C.

(c) Reactivation elementary step. This is achieved by release of the replica from the complex at the final stage of the elongation process and further rearrangement of the inactivated complex so that the enzyme binds at the active end of the template strand.

We shall follow ref. 3 in order to implement an adequate reaction scheme for the exponential growth phase but we shall make a major modification concerning the overall reactivation process: the effective rate constant associated with this process will be adjusted according to experimental evidence [9] and will prove to be two orders of magnitude smaller than the average rate constant for the polymerization elementary steps which constitute the elongation process.

Let I_1 denote the free RNA and the first replication complex which appears before the first phosphodiester bond is formed. There is no ambiguity in regarding both species as indistinguishable. The deterministic drifts for the elongation process correspond to the $n-1$ elementary steps

$$I_1 \xrightarrow{k_1} I_2 \xrightarrow{k_2} \dots \rightarrow I_{n-1} \xrightarrow{k_{n-1}} I_n \tag{1}$$

Where I_j is an elongation intermediate complex and the total template concentration (free and complexed) is

$$X_s = \sum_{l=1}^{n-1} [I_l] + 2[I_n] \tag{2}$$

We shall assume that the rate constant for each polymerization step is identical and given by:

$$k_j = k = k_P \frac{[S_0]}{[S_0] + K_S} \tag{3}$$

where k_P is the average rate constant for formation of a phosphodiester linkage ($k_P = 5\ s^{-1}$); K_s is the average substrate association equilibrium constant to be adjusted according to experimental data on induction periods and $[S_0]$, the effective substrate concentration. For the sake of simplicity, a palindromic RNA is assumed. Given the specific complementarity pairing which governs the replica formation, this simply means that the replica and template correspond to an identical strand. The necessary and sufficient conditions for a strand to be palindromic are: (a) n is even and (b) the nucleotides in the strand are complementary as we begin examining the strand from both ends simultaneously and move towards the center.

We shall introduce an effective reactivation step which comprises replica release and rebinding of the enzyme to the active 3′-end of the template. Moreover, we shall assume that the process given by:

$$I_n \xrightarrow{k_n} I + I_1 \tag{4}$$

is rate-determining. This is a realistic assumption for the length of RNA considered in this work (cf. refs. 1 and 2): 120 nucleotides. We shall assign the value $6 \times 10^{-2}\ s^{-1}$ to k_n following ref. 9 and our calculations on induction periods will reveal that this value is appropriate.

A detailed discussion of the mass-action kinetics governing the elongation and reactivation processes can be found in refs. 2 and 3. The validity of the Michaelis-Menten approximation in the polymerization steps is warranted by the very short lifetime of the complexes for bonds of the phosphodiester linkage [2,3].

The normal kinetic modes of the system will be obtained by taking into account the separation of relaxation time scales. The dominating mode is the overall template concentration (for free and complexed template), $[I_0]$. Once the system has been reduced to a Poincaré-Jordan normal form, where the subordinated modes are separated from the dominating mode although they may remain coupled to each other, the onset of a CM is given by the functional dependence of relaxing modes to the exponential growth of $[I_0]$. To a first approximation, this dependence is given by the adiabatic approximation familiar from standard chemical kinetics (see, for example, ref. 6).

The implementation of a stochastic CM approach for extremely low initial template concentration (1–10 strands per ml) is only valid if the fluctuations can account for some essential kinetic features. Below [NTP] = 0.2 mM, the fluctuations must be so small that the induction periods for overcoming the kinetic barrier are not reproducible under laboratory conditions. On the other hand, for [NTP] above 0.3 mM, they should be able to surmount the kinetic barrier and the predictions should reproduce the experimental lag times.

In this respect fig. 1 is revealing: The fluctuations are negligibly small for $[I_0]$ greater than 10^3

strands/ml, thus it is expected that a deterministic kinetic approach would suffice to describe the phenomenology in this regime (cf. ref. 2). On the other hand, for $[I_0]$ in the range $1-10^2$ strands/ml, the stochastic effects are conspicuous for [NTP] above 0.2 mM and negligibly small for [NTP] of the order of 0.2 mM or less. These facts are qualitatively in accord with the statements in the previous paragraph but a considerable amount of work is required to make the agreement precise.

3. Center manifold theory

In many instances, dissipative systems present a phase space contraction due to the statistical enslavement of some rapidly relaxing normal modes to the subordinating excited modes [4,5,7,8]. Such cases are best described by the center manifold (CM) approach which introduces the idea that the statistical subordination can be accounted for by restricting the probability density to a narrow strip along the CM. The CM is a locally attractive and locally invariant surface in phase space determined by a functional dependence of the relaxing degrees of freedom ($X_{f,j}$) to the subordinating modes ($X_{s,i}$). The latter thus become the CM coordinates. The width of the strip depends on the position on the CM. In this formulation, the object of interest is the probability density function denoted $P = P(X_s, X_f, t)$ where X_s is the vector of subordinating modes and X_f, the vector of enslaved degrees of freedom. The basic ansatz in CM theory is that we can factorize P in the following way:

$$P = Q_s(X_s, t) \times Q_f(X_f \mid X_s) \tag{5}$$

In our particular case of interest, we have $X_s = X_s = [I_0]$, the overall template concentration, and Q_s has a deterministic drift given by the exponential growth of $[I_0]$. The conditional probability factor $Q_f(X_f \mid X_s)$ accounts for the adiabatic following or statistical subordination of the relaxing kinetic degrees of freedom [6].

The induction periods for the onset of a macroscopically detectable template amplification, i.e., for the onset of the exponential phase of replication, are dependent on the following factors:

(a) The average correlation, $\bar{C}$, for the rate fluctuations in the normal rapidly relaxing kinetic modes.

(b) The Gaussian width w for Q_f about the CM.

(c) The kinetic barrier to be overcome by the fluctuations.

The dependence of the induction period on the nucleoside triphosphate concentration, [NTP], is crucial if we hope to reproduce the experimental results [1,2]. In order to derive this dependence theoretically, we shall proceed according to the following plan:

(i) The starting point is a generic Fokker-Planck (FP) equation for P corresponding to a reaction scheme for RNA replication with an adjustable effective rate constant for the rate-determining reactivation process of replica release.

(ii) Making use of eq. 1, we integrate the FP equation for P along the CM, i.e., with respect to the relaxing degrees of freedom. Through this process we obtain a smeared FP equation.

(iii) Introduce scaling relations among the kinetic parameters together with $\bar{C}$ and w in order to reduce the resulting equation to an adequate FP equation for Q_s. By adequate we mean that the smeared FP equation should actually represent the restriction of the system to the CM, in other words, the deterministic drift in the smeared FP equation must yield the exponential growth of the total template concentration. This plan, once carried out, provides the required information regarding the dependence of $\bar{C}$ on [NTP] and allows us to calculate the size of fluctuations about the CM for [NTP] within the experimental range 0.1–0.5 mM and $[I_0]$ in the range $1-10^3$ molecules/ml.

We shall follow standard notation and denote by X the concentration vector (for each species, the concentration is given by the number of molecules per unit volume) and by ν_{kj} ($k = 1, \ldots, M$; $j = 1, \ldots, N$), the stoichiometric coefficient of the j-th species in the k-th elementary step. Let T be the transformation associated with the local CM. This transformation is defined by:

$$T(t) = T = (b_{ij}),\ T: X \to Y = \begin{pmatrix} X_s \\ X_f \end{pmatrix} \tag{6}$$

where X_s is the vector of the excited modes and X_f, that of the subordinated degrees of freedom.

This means that the Jacobian matrix in the Y representation, TJT^{-1}, is in Poincaré-Jordan normal form, or, in other words, that the excited modes and the subordinated degrees of freedom have been separated. The term J denotes the Jacobian matrix in the X representation.

If C represents the fluctuation covariance for the original system, we shall show that the fluctuation covariance in the Y representation is given by

$$\tilde{C} = T(TC)^t \tag{7}$$

where

$$C = \left(\langle f_i(t) f_j(t)\rangle\right) \tag{8}$$

Each replica in the ensemble is defined by specifying the following small parameters:

(i) The Gaussian width of the probability density about the CM.

(ii) The real part of the eigenvalues of J which are on the right-hand side of the imaginary axis.

(iii) The scaling factor for the covariance matrix for internal fluctuations. This factor will be denoted L^{-1}.

Some explanation is in order: the far-from-equilibrium fluctuations which trigger the onset of the CM cannot be scaled with the thermodynamic volume [7], rather, the volume to which they are confined is a virtual volume, W, with $W/V = L^{-1}$. The scaling factor L^{-1} is introduced to display explicitly the relative size of the terms which appear in the smeared FP equation for Q_s, leading to an equation of continuity for the flow of probability about the CM.

We now need to prove eq. 7 in order to ensure that if C is of order L^{-1}, then $\tilde{C}$ is also of order L^{-1}.

The equation for the deviation of the ensemble average $x = X - \langle X\rangle$ is

$$\dot{x} = J(t)x + f(t) \tag{9}$$

Or, alternatively, denoting $y = Y - \langle Y\rangle$:

$$\dot{y} = (TJT^{-1})y + Tf(t) \tag{10}$$

Thus, eq. 7 can be readily demonstrated by noting that

$$\begin{aligned}\tilde{C}_{ij}\delta(t-t') &= \langle (Tf)_i(t)(Tf)_j(t')\rangle \\ &= \left\langle \left(\sum_{n=1}^{N} b_{in} f_n(t)\right)\left(\sum_{m=1}^{N} b_{jm} f_m(t')\right)\right\rangle \\ &= \sum_{n,m=1}^{N} b_{in} b_{jm} C_{nm}(t)\delta(t-t') \end{aligned} \tag{11}$$

Thus, the explicit form of the covariance in the Y representation is

$$\tilde{C}_{ij}(t) = L^{-1} \sum_{k=1}^{M} \sum_{n,m=1}^{N} \nu_{ki}\nu_{kj} b_{in} b_{jm} (\nu_k^+ + \nu_k^-) \tag{12}$$

where ν_k^+ and ν_k^- designate the forward and reverse rates for the k-th elementary step, respectively.

The strength of internal fluctuations is determined by the fact that we should allow for a continuous flow of probability about the CM.

To fix the notation, we shall denote the number of subordinating modes by S and the number of enslaved degrees of freedom by F.

The FP equation for P integrated along the CM has the general form:

$$\begin{aligned}&\int_{\text{c.m.}} \partial_t P(X_s, X_f, t)\, \mathrm{d}X_f \\ &= \int_{\text{c.m.}} \Bigg(\sum_{i=1}^{S} \partial_{X_{s,i}} \left\{ \left[\dot{X}_{s,i} - (T_f)_{s,i} \right] P \right\} \\ &\quad + \sum_{j=1}^{F} \partial_{X_{f,j}} \left\{ \left[\dot{X}_{f,j} - (T_f)_{f,j} \right] P \right\} \\ &\quad + \sum_{ii'=1}^{S} \tfrac{1}{2} \tilde{C}_{ii'} \partial^2_{X_{s,i} X_{s,i'}} P \\ &\quad + 2 \sum_{i=1}^{S} \sum_{j=1}^{F} \tfrac{1}{2} \tilde{C}_{ij} \partial^2_{X_{f,i} X_{f,j}} P \\ &\quad + \sum_{i,j'=1}^{S} \tfrac{1}{2} \tilde{C}_{jj'} \partial^2_{X_{f,j} X_{f,j'}} P \Bigg)\, \mathrm{d}X_f . \end{aligned} \tag{13}$$

In order to carry out this integration, we need the explicit form of the factor Q_f:

$$Q_f = Q_f(X_f \mid X_s) = \prod_{j=1}^{F} (g_j/\pi)^{1/2} \exp\left\{-g_j\left[X_{f,j} - \bar{F}_j(X_s)\right]^2\right\} \tag{14}$$

where $\bar{F}_j(X_s) = \tilde{X}_{f,j}$ represents the CM equation, the Gaussian width is given by $w_j = (2g_j)^{-1/2}$ and the coefficients g_j must be determined so that the time evolution of the probability density Q_s corresponds to the exponential growth of the total template concentration.

We shall introduce the following scaling relation:

$$\tau = L^{-1/3}t \tag{15}$$

and denote $\tilde{P}(X_s,\tau) = Q_s(X_s,t)$.

Thus, the smeared CM-reduced FP equation has the general form:

$$\partial_\tau \tilde{P} = \sum_{i=1}^{S} \partial_{X_{s,i}}\left\{\left(\dot{X}_{s,i} - (Tf)_{s,i}\right)\tilde{P}\right\} + \tfrac{1}{2}\sum_{i=1}^{S} \tilde{C}_{s,is,i}\partial^2_{X^2_{s,i}}\tilde{P} \tag{16}$$

4. Center manifold kinetics

The specific form of the reduced FP CM equation is:

$$\partial_\tau \tilde{P} = \partial_{[I_0]}\left(\lambda_n[I_0]\tilde{P}\right) + \tfrac{1}{2}C_{ss}\partial^2_{[I_0]^2}\tilde{P} \tag{17}$$

where λ_n is the damping constant (in this case positive) corresponding to the subordinating eigenmode. The eigenvalues can be obtained following the canonical diagonalization procedure for the Jacobian of the kinetics given by eqs. 1–4. This process has been described extensively in ref. 3. In our case, some complications arise in the computation, since not all the effective rate constants for the elementary steps are equal, as in the model for the exponential growth phase presented in ref. 3. In a realistic model, the reactivation step has an effective rate constant two orders of magnitude smaller than the elongation rate constants. Nevertheless, since n is large, we find the following asymptotic relation following the computation given in ref. 3:

$$\lambda_n = \sum_{j=1}^{n} \frac{\left(k^j k_n^{n-j}\right)^{1/n}}{(n-j+1)}\left(2^{1/n}-1\right)^{n-j+1} \tag{18}$$

The reduction of the general equation (eq. 13) to yield eq. 17 requires not only making use of 14 but also introducing certain scaling relations in order to display the relative size of the terms which appear upon integration along the CM. If a variable generically denoted by X is of order L^a, then we shall represent X as $X = L^a X_1$. We now demonstrate that eq. 13 reduces to eq. 17. If we adopt the following scaling of small parameters:

$$\begin{aligned} &\lambda_n = O(L^{-1/3}) \\ &\tau = L^{-1/3}t \text{ (dimensionless time)} \\ &\bar{C} = O(L^{-1}) \\ &\bar{w} = \text{averaged Gaussian width} = O(L^{-1/2}) \end{aligned} \tag{19}$$

The scaling relations are introduced, since we need to show that by making use of the factorization of P in a time-dependent factor and a conditional probability, we obtain a reduced FP equation whose deterministic drift is given by the exponential growth of the total template concentration. This reduced FP equation must be obtained upon integration of the initial FP equation for P along the CM. This means that certain terms should be neglected in the integrated equation for $\tilde{P}$ as being higher order in L^{-1}. The elimination of such terms should allow for a continuous flow of probability about the CM, i.e., we end up with a diffusion pressure about the CM given by the fluctuation correlations of the subordinating mode only.

Thus, making use of eqs. 13 and 14, we obtain:

$$\partial_t \tilde{P} = \partial_{X_s}\left\{\left(\tilde{k}_1 L^{-1/3}X_s + O(L^{-1})\right)\tilde{P}\right\} - \sum_{m=1}^{n-1}\left\{\left(\tilde{k}_1 L^{-1/3}X_s + O(L^{-1})\right)\frac{(g_m)_1}{2g_m}\tilde{P}\right\} - \sum_{m=1}^{n-1}\left(\tilde{C}_{mm}\right)_1 (g_m)_1 \tilde{P} + \left(L^{-1}\tilde{C}_{ss}/2\right)$$

$$\times\left\{\left(\partial^2_{X_s X_s}\tilde{P}+\sum_{m=1}^{n-1}\frac{(g_m)_1}{g_m}\partial_{X_s}\tilde{P}\right)\right.$$

$$\left.+\sum_{m=1}^{n-1}\left(\frac{(g_m)_1^2}{4g_m}+O(L^{-1/3})\right)\tilde{P}\right\} \qquad (20)$$

where $\tilde{C}_{mm}$ is short for $\tilde{C}_{f,m,f,m}$ and $\tilde{k}_1=(\lambda_n)_1$.

This equation reduces to eq. 17 to order $L^{-1/3}$ if and only if the probability density is distributed about the CM according to the formula:

$$g_m=\left\{\lambda_m/\left(2\tilde{C}_{mm}\sqrt{M}\right)\right\}[I_0]^2 \qquad (21)$$

where $M=1\ s^2$ and

$$\lambda_m=\sum_{j=1}^{n}\frac{\left(k^j k_n^{n-j}\right)^{1/n}}{n-j+1}$$

$$\times\left\{2^{1/n}\cos(2\pi m/n)-1\right\}^{n-j+1} \qquad (22)$$

The induction period is given by [10]:

$$T_{\text{ind}}=\frac{W}{\bar{C}}\exp(B/\bar{C}) \qquad (23)$$

where $\bar{C}$ denotes the average fluctuation correlation, B the size of the kinetic barrier and W is given by:

$$W=\int_{X_f-\tilde{X}_f(X_s)=0}^{w}Q_f\left(X_f\mid X_s(t=0)\right)\,dX_f \qquad (24)$$

The size of the barrier B will be regarded as an adjustable parameter and also the equilibrium constant K_s. The value given in ref. 3 for the latter parameter will not be adopted, since the experimental set-up discussed in ref. 1 is different from that of ref. 3 with respect to the RNA species involved. The remaining kinetic parameters are as follows [1–3]: n = length of the chain = 120; k_p = 5 s^{-1}; [NTP] as specified in the legend to fig. 1. The Michaelis-Menten constant K_s is obtained through making use of the working equations, eqs. 14, 19, 21 and 23 and using as input the following experimental data [1,2]:

$T_{\text{ind}}([\text{NTP}]=0.5\ \text{mM})=35\ \text{min}$

$T_{\text{ind}}([\text{NTP}]=0.20\ \text{mM})=350\ \text{min}$

A straightforward calculation gives:
$B=4.40\times10^3$ molecules $ml^{-1}\ s^{-1}$
$K_s=6.10\times10^{-3}$

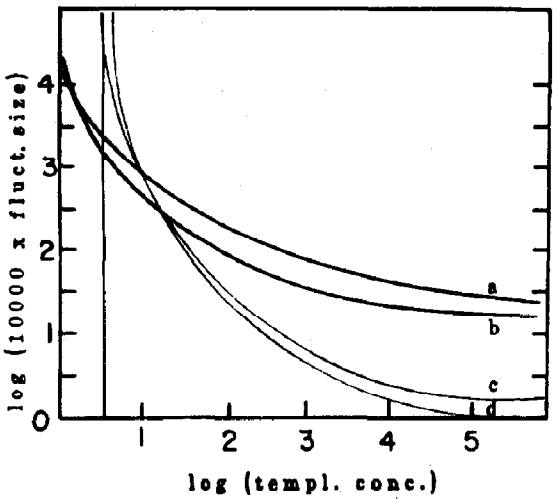

Fig. 1. Center manifold calculation of far-from-equilibrium fluctuations in template concentration for the experimental set-up presented in ref. 1. The abscissa is given on a logarithmic scale, the template concentration being in number of molecules per cm^3. The ordinate is also given on a logarithmic scale with the fluctuation size being in number of molecules per cm^3. (Curve a) [NTP] = 0.10 mM, (curve b) [NTP] = 0.20 mM, (curve c) [NTP] = 0.30 mM, (curve d) [NTP] = 0.50 mM.

Note that this last value is one order of magnitude larger than that given in ref. 3.

Since $K_s \gg [\text{NTP}]$, we have, from eq. 19: $\bar{C}\propto[\text{NTP}]^3$. Therefore, for $B \ll \bar{C}$ (which holds at [NTP] of the order of 0.5 mM, see fig. 1) we obtain:

$$T_{\text{ind}}\propto\frac{W}{[\text{NTP}]^3}\left\{1+\frac{B}{[\text{NTP}]^3}+\frac{B^2}{[\text{NTP}]^6}+\ldots\right\} \qquad (25)$$

This derivation is in very good agreement with the empirical law: $T_{\text{lag}}\propto[\text{NTP}]^{-2.75}$.

Table 1

[NTP] dependence for induction periods

[NTP] (mM)	T_{ind} (min)	
	Theoretical	Experimental
0.50	46	35
0.30	159	120
0.20	405	350
0.15	~ 700	∞
0.10	~ 2800	∞

Making use of the results displayed in fig. 1, we can now calculate the lag times theoretically as shown in table 1. We observe that the values lie within the region of scattering corresponding to the experimental uncertainty and that they correlate well with the experiment [1,2].

5. Conclusion

Very few treatments following the so-called 'thermodynamics of irreversible processes' have effectively attempted to evaluate the intensity of far-from-equilibrium fluctuations. Such approaches are essentially based upon Onsager's essay of 1931 on the near-equilibrium reciprocity laws [11]. Making use of a stochastic CM reduction, one can show that extrapolations from the near-equilibrium realm, introducing a Langevin source into the phenomenological equations, are of no avail whenever there is an adiabatic following of subordinated macroscopic degrees of freedom [4]. Thus, in the transition to a convective roll pattern for a Rayleigh-Bénard cell, the strength of far-from-equilibrium fluctuations is three orders of magnitude greater than their equilibrium counterparts.

In the dynamics of RNA replication catalyzed by Qβ-replicase 'contaminated' with traces of template, a similar situation holds. For instance, at an initial concentration of 10^2 template strands/ml, we find again that the fluctuations in total template concentration are three orders of magnitude greater than their equilibrium counterparts. The laws variance $= O(Z^{1/2})$ (with Z = total number of template strands) breaks down. This is indicated by eq. 21.

Upon inspection of fig. 2, we can derive the initial template concentration which yields an experimentally measurable induction period [1,2]. The results listed in table 1 were obtained via extrapolation to an initial concentration of 1 strand/ml and evidently fit satisfactorily the experimental findings. Fig. 2 (curve a, upper branch) reveals that such a procedure is correct for a realistic NTP concentration of 0.5 mM if we compare vis-a-vis the corresponding experimental result shown in table 1.

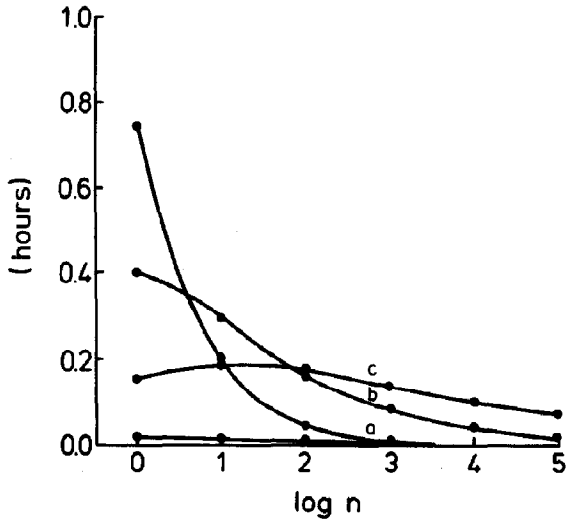

Fig. 2. Induction period as a function of the initial template concentration. Abscissa: n = number of template strands/ml; ordinate: induction periods given in fractions of 1 h. (Curve a) [NTP] = 0.5 mM, upper branch: $K_s = 6.1 \times 10^{-3}$, lower branch: $K_s = 2.0 \times 10^{-4}$. [3]. (Curve b) [NTP] = 0.7 mM, $K_s = 6.1 \times 10^{-3}$. (Curve c) [NTP] = 1.0 mM, $K_s = 6.1 \times 10^{-3}$.

The extreme sensitivity of the rate self-correlation, $\bar{C}$, to the value of the effective substrate dissociation equilibrium constant, K_s, manifests itself in the difference between the two branches for curve a in fig. 2: the K_s value from ref. 3 is one order of magnitude lower than that for the experimental set-up in ref. 2, as determined in this work, and yields unrealistically low values of T_{ind}. Thus, the relevance of our treatment in deriving the equilibrium constant K_s becomes apparent.

It should be stressed once more that the critical template concentrations obtained from fig. 2 should be interpreted as effective or virtual concentrations and not as the actual concentrations for the reacting mixture. This is so since the rate-fluctuation correlation $\bar{C}$ does not scale with the inverse of the thermodynamic volume, as is the case near equilibrium. Instead, the fluctuations are local in nature [10], i.e., they are confined to a virtual volume and their correlation scales with the control rate parameters of the system as determined by eq. 19. A self-consistent derivation of virtual volumes, in the spirit of ref. 10, is very involved and will be given in a forthcoming paper.

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