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The Origin of the RNA World: A Kinetic Model

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Received: July 24, 1998; In Final Form: January 11, 1999

The aims of this paper are to propose, construct, and analyze microscopic kinetic models for the emergence of long chains of RNA from monomeric β-D-ribonucleotide precursors in prebiotic circumstances. Our theory starts out from similar but more general chemical assumptions to those of Eigen,[1] namely, that catalytic replication can lead to a large population of long chains. In particular, our models incorporate the possibility of (i) direct chain growth, (ii) template-assisted synthesis, and (iii) catalysis by RNA replicase ribozymes, all with varying degrees of efficiency. However, in our models the reaction mechanisms are kept "open"; we do not assume the existence of closed hypercycles which sustain a population of long chains. Rather it is the feasibility of the initial emergence of a self-sustaining set of RNA chains from monomeric nucleotides which is our prime concern. Moreover, we confront directly the central nonlinear features of the problem, which have often been overlooked in previous studies. Our detailed microscopic kinetic models lead to kinetic equations which are generalisations of the Becker−Döring system for the stepwise growth of clusters or polymer chains; they lie within a general theoretical framework which has recently been successfully applied to a wide range of complex chemical problems. In fact, the most accurate model we consider has Becker−Döring aggregation terms, together with a general Smoluchowski fragmentation term to model the competing hydrolysis of RNA polymer chains. We conclude that, starting from reasonable initial conditions of monomeric nucleotide concentrations within a prebiotic soup and in an acceptable timescale, it is possible for a self-replicating subset of polyribonucleotide chains to be selected, while less efficient replicators become extinct.

1. Introduction

Since the early 1970s, an impressive body of theoretical and experimental work has been accumulating which supports the so-called "RNA world" view.[2,3] According to this picture, the central dogma of molecular biology, that "DNA makes RNA makes protein", is replaced with the view that "in the beginning" both the genetic material and the (enzymatic) catalysts were comprised of RNA. Altman and Cech won the 1989 Nobel prize in chemistry for demonstrating that RNA does indeed have catalytic powers and that there are naturally occurring enzymes made of RNA, now known as ribozymes.[4] According to this standpoint, the problem of the evolution of life thus divides into two parts: (i) where did the RNA world come from, and (ii) how did that develop into the world we know today involving DNA, RNA, and proteins? Both of these questions are formidable, but whereas the second is widely appreciated, the first is more easily overlooked.[5]

This paper aims to address the first of these two questions.

When the issue is considered carefully, it is apparent that it is by no means straight forward to provide a convincing answer. On the one hand, there is a lack of evidence for the spontaneous formation of the β-D-ribonucleotide monomers that comprise RNA under plausible prebiotic conditions (the pyrimidine-containing compounds in particular have failed to appear in all experiments to date); it remains unclear how nucleotides of the right chirality appeared and what led to stereospecific 3′−5′ polymerization; and finally no feasible prebiotic RNA replicator has yet been found. However, progress is being made in this direction; for example, it is known that chemically activated nucleotides can form oligomeric chains of 20−40 nucleotides in length in the absence of templates.[6] It seems at least reasonable to be optimistic on this score and to assume that in the not too distant future truly autocatalytic RNA molecules will be made.

On the other hand, due to the distinct information content carried by different nucleotide base sequences within RNA polymers, the problem has a combinatorial complexity which puts it into the class of NP hard problems in the sense of algorithmic complexity theory.[4] In particular, because there are four bases within RNA (A, G, C, and U), a linear RNA polymer of length N monomers can exist as 4^N chains with different base

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sequences. Not only does this preclude numerical analysis of the problem for anything other than very small values of N, it has been used prima facie as an implausibility argument against the very possibility of producing self-replicating RNA molecules from a prebiotic soup comprised initially of nucleotide monomers. According to this argument, to produce a significant concentration of any one self-replicating RNA molecule—assuming this to be chemically feasible—would have required a greater mass of molecules in the prebiotic soup than is available from the mass of the Earth.[7] Such an argument may be rendered invalid if the kinetics of the system are highly nonlinear, as indeed they are in the models we present here.

Putting aside the several outstanding chemical questions concerning the realization of actual examples of self-replicating RNAs on the assumption that these can and will eventually be achieved, the aim of the present paper is to present and analyze kinetic models of RNA chain formation and self-replication in prebiotic conditions. The central purpose of this work is to assess the feasibility of achieving viable concentrations of self-replicating RNA polymers from plausible estimates of the physicochemical parameters and conditions which most likely pertained under prebiotic conditions. (Although for the purposes of the study presented in this article, only the RNA worldview is considered, we wish to point out that there is by no means a consensus on this question; for an overview of different standpoints see Fleischaker et al.[8])

One part of our paper is concerned with proposing detailed microscopic kinetic models of RNA formation and self-replication; these models are loosely based on what we refer to as the so-called "Becker−Döring" assumptions of classical nucleation theory. The major extensions and generalizations of this theory which are needed to faithfully model the complexity of the current situation lead to equations which, as they stand, are far too complex to analyze theoretically or numerically. Moreover, the chemistry which these rate processes describe is far too detailed to be of direct use to experimentalists. Hence we formulate a "coarse-graining" reduction aimed at overcoming the combinatorial complexity inherent in these systems, and which simplifies the kinetic equations so that useful analysis and comparisons with experiment are possible. This reduction procedure is a major theme of the present paper.

In some theoretical respects our work is close to that of Eigen[1,9−12] and Nuño et al.[13−18] in that we are modeling the formation of RNA as a complex sequence of chemical reactions which proceed via catalytic and noncatalytic pathways. The main differences between our approach and that of both Nuño and Eigen are that our reaction networks are all *open-ended* rather than closed cycles ("hypercycles") and that we address the full nonlinearity of the kinetic equations involved.

Moreover, from a chemical perspective our work is mainly aimed at answering a significantly different question to that posed by Eigen and Nuño et al. Their work explains how large concentrations of long chains cooperate in order to maintain unexpectedly high concentrations when one might expect only very few, if any at all, to be present. By contrast, our work deals with an earlier stage of the process, namely how long chains are formed from a system which initially has only the basic building blocks (monomers) present. The temporal behavior of our models is, in fact, very similar to that shown in Figure 16 of Eigen.[1]

Because the full problem we are addressing is mathematically very complex, we approach it in a sequence of stages within the different sections of the paper as now outlined. In section 2, a highly simplified but instructive heuristic model is described

and analyzed. This idealized "toy" model shows how the various essential rate processes involved, when combined together, produce qualitatively the type of behavior we would hope to see in the more realistic and much more complex models of RNA formation and self-replication we later construct.

The remainder of the paper presents and analyzes various more detailed models, based on the Becker−Döring equations for step-wise growth. The main point to make here is that sections 3 and 4 are concerned respectively with detailed and coarse-grained models of RNA chain formation and growth in the absence of hydrolysis (which breaks up growing chains), whereas sections 5 and 6 include this important process and demonstrate the dramatic effect it has on maximal chain length expected. We then investigate under what circumstances some species become extinct and others dominate.

The models derived in sections 3 and 5 are vastly more complex than the original Becker−Döring equations,[19,20]—and indeed even more complex than the generalizations we have previously developed to describe micelle and vesicle self-reproduction,[21,22] as well as generalized nucleation processes[23]—since in the present work we attempt to keep track of the information contained in the RNA sequences by noting the order of addition of nucleotides. This then enables us to incorporate the effects of template-assisted and ribozymic catalysis of chain formation and replication. The paper closes with a discussion in section 7 while an appendix describes a model similar to the one developed in section 3 but incorporating more complex kinetic processes.

2. A Simple Heuristic Model

In this section we present a very simple heuristic model of RNA formation and self-replication which contains the basic and essential properties of slow growth by an uncatalysed reaction mechanism, fast growth due to catalysis and error-prone replication, and we show that it produces results consistent with our intuition, namely, a long induction time, after which large concentrations of products are formed.

We assume there is a steady source of nucleotides from which to build chains; then, with no catalysis and no errors during chain polymerization (growth), we expect an exponential growth in concentration (u) as described by

$$\frac{\partial u}{\partial t} = u \qquad (2.1)$$

Now we shall allow u to depend also on the composition of the growing RNA chains (that is, on the sequence of nucleotide bases of which they are comprised), which we denote $\mathbf{x}$. Errors in replication of $\mathbf{x}$ lead to the creation of chains with similar composition, and are thus close to $\mathbf{x}$ in this "sequence space"; this can be thought of as a diffusive process in sequence space. Thus we propose the following partial differential equation

$$\frac{\partial u}{\partial t} = D\nabla^2_{(\mathbf{x})} u + u + \alpha u^2 \qquad (2.2)$$

On the right hand side, the $D\nabla^2_{(\mathbf{x})} u$ term describes the diffusive spread of material from one type of composition to another via imperfect replication, while the term αu^2 models catalytic replication, whereby if some species ($\mathbf{x}$) exists, its rate of production will depend on its own concentration $u(\mathbf{x}, t)$. Equation 2.2 has the form of a nonlinear reaction-diffusion equation; indeed, equations of this general structure are well known and their properties have been much studied in the context of a

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mathematical theory of explosive chemical reactions (thermochemical runaway); see, for example, work by Dold.[24]

Our first step is to show that there is a growing solution which is independent of the information in the chains, $u = f(t)$, independent of $\mathbf{x}$. From eq 2.2, this amounts to solving the ordinary differential equation (ODE)

$$\dot{f} = f + \alpha f^2 \qquad (2.3)$$

which has a solution $f(t) = f_0 e^t / (1 + \alpha f_0 (1 - e^t))$. This expression for $f(t)$ blows up in a finite time, at $t_c = \log(1 + 1/\alpha f_0)$.

Now we show that the behavior predicted by this solution is unstable, in the sense that a perturbation of the solution which depends on the information variable $\mathbf{x}$ (i.e., on the nucleotide sequence of the chain) has an even faster growth rate. We put $u(\mathbf{x}, t) = f(t) + h(\mathbf{x}, t)$, with $h \ll f$. We linearize about $f(t)$ and find that $h(\mathbf{x}, t)$ satisfies

$$\frac{\partial h}{\partial t} = D\nabla^2_{(\mathbf{x})}h + h + \frac{2\alpha f_0 e^t h}{1 + \alpha f_0 (1 - e^t)} \qquad (2.4)$$

which can be solved by separation of variables, using $h(\mathbf{x}, t) = X(\mathbf{x})\,T(t)$:

$$\frac{T'(t)}{T(t)} - 1 - \frac{2\alpha f_0 e^t}{1 + \alpha f_0 (1 - e^t)} = \frac{D\nabla^2_{(\mathbf{x})}X(\mathbf{x})}{X(\mathbf{x})} = -\mu \qquad (2.5)$$

In the simplest case, where we take a one-dimensional information space $\mathbf{x} \equiv x \in \mathbf{R}^1$, the shape in x-space of the variation $h(\mathbf{x}, t)$ is $\sin(x\sqrt{\mu/D})$, $\cos(x\sqrt{\mu/D})$, indicating an increase in the concentration for some x values and a decrease in others (relative to the average concentration $f(t)$). In fact, this formulation is valid whatever dimension the space of all $\mathbf{x}$'s is taken to have. The temporal evolution of the perturbation is unaffected by $X(\mathbf{x})$, and is given by

$$T(t) = \frac{B(1 + \alpha f_0)^2 e^{(1-\mu)t}}{(1 + \alpha f_0 (1 - e^t))^2} \qquad (2.6)$$

This function increases more rapidly than f for t sufficiently close to t_c (if $\mu > 1 + 2\alpha f_0$ then h at first decreases and then increases, blowing up at $t = t_c$).

In summary, the solution to the heuristic eq 2.2 we have derived is

$$u(\mathbf{x}, t) \sim \frac{f_0 e^t}{1 + \alpha f_0 (1 - e^t)} + \frac{B(1 + \alpha f_0)^2 X(\mathbf{x}) e^{(1-\mu)t}}{(1 + \alpha f_0 (1 - e^t))^2} \qquad (2.7)$$

which, for times near blow-up ($t \lesssim t_c = \log(1 + 1/\alpha f_0)$), shows that a system with modulations in composition ($\mathbf{x}$) has faster growth rates than a system without such dependency, indicating that some species will be preferred over others on the basis of their specific chemical composition (information content).

Although the very simple model presented and analyzed in this section displays some rather unphysical behavior, such as the presence of a singularity corresponding to the development of infinite concentrations of RNA chains in finite time, the very fact that it is possible to produce very high concentrations of products starting from arbitrarily low concentrations of reactants is encouraging for our quest to account for the origin of the RNA world. Our next step is to construct and analyze the dynamical behavior of more realistic, and of course much more complicated, models of RNA formation and self-replication. It

is possible to tame the singularities which develop in these more realistic models; as we shall demonstrate, one means of achieving this is by incorporating hydrolysis into such schemes, which causes the breakdown of longer chains (sections 5 and 6).

3. Models of RNA Polymerization in the Absence of Hydrolysis

Our aim now is to begin to model the chemical processes involved in the formation and replication of RNA. We will denote the four nucleotides bases A, C, G, and U by N_i with $i = 1, 2, 3, 4$; and oligomeric ribonucleotide sequences by C_r^γ, where r signifies the number of bases in the sequence and γ denotes the particular order in which they occur.

The main reactions that such chains can undergo are (**i**) the basic Becker–Döring rate processes controlling chain growth

$$C_r^\gamma + N_i \underset{\text{slow}}{\rightleftharpoons} C_{r+1}^{\gamma + N_i} \qquad (3.1)$$
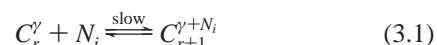
(**ii**) template-based chain synthesis (a form of catalysis mediated by Watson–Crick base pairing of ribonucleotides on complementary chains)

$$C_r^\gamma + N_i + C_s^\theta \underset{\text{fast}}{\rightleftharpoons} C_{r+1}^{\gamma + N_i} + C_s^\theta \qquad (3.2)$$
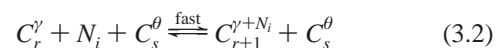
Some nucleotide sequences C_r^γ are better "replicators" (templates) than others, by virtue of base-pairing effects. In particular we expect that the complementary chain to γ (in the sense of Watson–Crick hydrogen-bonded base pairs), which we shall denote γ^*, will be an extremely effective template for the production of γ; (**iii**) "poisoning" or inhibition, for example, by tightly-bound duplex formation

$$C_r^\gamma + C_s^\theta \rightleftharpoons P_{r,s}^{\gamma,\theta} \qquad (3.3)$$

In the present paper, we have not made any serious attempts to incorporate inhibition into our detailed models; (**iv**) hydrolysis, whereby a long chain is split into two shorter chains. Chemically this corresponds to the reaction $C_{r+s}^{\gamma+\theta} \rightarrow C_r^\gamma + C_s^\theta$. This has the form of a general fragmentation process as modelled by the Smoluchowski equations,[25] a mechanism which increases the number of chains but reduces the average chain length; (**v**) enzymatic replication (replicase ribozymal activity), where a third chain aids the growth of a chain which is already in close contact with another chain acting as a template

$$C_r^\gamma + N_i + C_{r+k}^{\gamma^*+\theta^*} + C_s^\xi \rightleftharpoons C_{r+1}^{\gamma + N_i} + C_{r+k}^{\gamma^*+\theta^*} + C_s^\xi \quad (3.4)$$
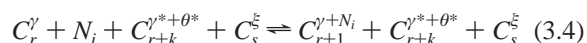
here the combination of γ with N_i is a subsequence of the chain $\gamma + \theta$ (that is, $\gamma + N_i \subset \gamma + \theta$) while C_s^ξ plays the part of a replicase ribozyme. Needless to say, some of these replicases will have much higher efficiency than (most) of the others.

We note in passing that, according to Eigen et al.,[12] the only efficient self-replicators have $r \approx 70$ (longer chains have copying errors too high to guarantee accurate replication). Our model ultimately confirms that there is a critical length, beyond which chains cannot self-replicate with sufficient accuracy to remain viable. The manner in which this critical length depends on the self-replication error rate; the uncatalyzed growth rates and autocatalytic rates is given in section 6.2.

We use C_r^γ to denote the chain γ which has length r, and other chains which it may interact with will be denoted C_s^θ, whose sequence of bases is encoded in θ and has length s. We define a flux corresponding to the addition of each type of

monomer ($\{N_i\}_{i=1}^4 = \{A, C, G, U\}$) to each type of chain γ. Since there are two ends to each chain there are two growth points. The reactions are given by

$$C_r^\gamma + N_i \rightleftharpoons C_{r+1}^{\gamma+N_i}, \quad \text{and} \qquad N_i + C_r^\gamma \rightleftharpoons C_{r+1}^{N_i+\gamma} \quad (3.5)$$

The rates of these reactions will include effects arising from replicase ribozymal activity, as shown explicitly in the flux functions $J_r^{\gamma,N}$ below. The superscripts carry information on the exact sequences of bases, and of course the order of the bases is important: N_1 followed by γ is a different sequence to γ followed by N_1.

We start the mathematical modelling of these processes by considering only the reversible aggregation mechanisms. These all fall into the class of Becker−Döring processes of stepwise cluster growth. In the first part of this paper, we shall neglect the hydrolysis and the poisoning mechanisms in order to investigate in detail the primary growth mechanisms that such an already formidably complex system allows. Later on, we shall return to study the effect of hydrolytic fragmentation mechanisms (sections 5 and 6), postponing further consideration of inhibition for a subsequent publication.

The flux $J_r^{[\gamma,N_i]}$ denotes the rate of attachment of the nucleotide monomer (base) N_i to the right hand end of γ. Similarly $J_r^{[N_i,\gamma]}$ denotes the rate of attachment of the monomer N_i to the left hand end of γ. The processes described above mention three mechanisms for growth: an uncatalysed chain growth step, two template-based growth steps which lead to quadratic catalysis and a ribozymal replicase process which is cubic in the reactant concentrations. We take template-based chain growth as being error prone in that it is most effective when $\theta = \gamma^*$, but it will also affect the overall rate of aggregation when $\theta \neq \gamma^*$:

$$J_r^{[\gamma,N_i]} = \left(c_r^{[\gamma]} c_1^{[N_i]} - \frac{\bar{c}_r^{[\gamma]} \bar{c}_1^{[N_i]}}{\bar{c}_{r+1}^{[\gamma+N_i]}} c_{r+1}^{[\gamma+N_i]}\right)\left(\epsilon_{r,0}^{[\gamma,N_i]} + \alpha_r^{[\gamma,N_i]} c_{r+1}^{[\gamma+N_i]} + \right.$$
$$\left. \sum_\theta \chi_{r,s}^{[\gamma,N_i,\theta]} c_s^{[\theta]} + \sum_\theta \sum_{\substack{\xi: \\ \gamma+N_i \subset \xi}} \zeta_{r,s,k}^{[\gamma,N_i,\theta,\xi]} c_s^{[\theta]} c_{r+k}^{[\xi]}\right) \quad (3.6)$$

$$J_r^{[N_i,\gamma]} = \left(c_r^{[\gamma]} c_1^{[N_i]} - \frac{\bar{c}_r^{[\gamma]} \bar{c}_1^{[N_i]}}{\bar{c}_{r+1}^{[N_i+\gamma]}} c_{r+1}^{[N_i+\gamma]}\right)\left(\epsilon_{r,0}^{[N_i,\gamma]} + \alpha_r^{[N_i,\gamma]} c_{r+1}^{[N_i+\gamma]} + \right.$$
$$\left. \sum_\theta \chi_{r,s}^{[N_i,\gamma,\theta]} c_s^{[\theta]} + \sum_\theta \sum_{\substack{\xi: \\ N_i+\gamma \subset \xi}} \zeta_{r,s,k}^{[N_i,\gamma,\theta,\xi]} c_s^{[\theta]} c_{k+r}^{[\xi]}\right) \quad (3.7)$$

We use γ_1 to denote the left-hand end monomer of the chain γ, and γ_r for the right-hand end monomer; $\gamma - \gamma_r$ denotes the chain γ without the final (γ_r) monomer and $-\gamma_1 + \gamma$ the chain without its first element. When this information is included in the notation of a concentration or a rate constant, we shall place it as a superscript in square brackets to avoid any possible confusion with exponents. Using this notation, the kinetic equation for the concentration of the chain γ is then

$$\dot{c}_r^{[\gamma]} = J_{r-1}^{[\gamma_1,-\gamma_1+\gamma]} + J_{r-1}^{[\gamma-\gamma_r,\gamma_r]} - \sum_{i=1}^4 J_r^{[N_i,\gamma]} - \sum_{i=1}^4 J_r^{[\gamma,N_i]} - $$
$$\sum_\theta k_{r,s}^{[\gamma,\theta]} c_r^{[\gamma]} c_s^{[\theta]} - \sum_\theta k_{s,r}^{[\theta,\gamma]} c_r^{[\gamma]} c_s^{[\theta]} + $$
$$\sum_\theta m_{r,s}^{[\gamma,\theta]} p_{r,s}^{[\gamma,\theta]} - \sum_\theta m_{s,r}^{[\theta,\gamma]} p_{s,r}^{[\theta,\gamma]} \quad (3.8)$$

(the apparent duplication of terms being due to the assumption

that the model must be able to distinguish which end of the chain a monomer is added to).

These equations introduce the poisoned duplices $P_{r,s}^{\gamma,\theta}$ whose concentrations $p_{r,s}^{[\gamma,\theta]}$ satisfy the equation

$$\dot{p}_{r,s}^{[\gamma,\theta]} = k_{r,s}^{[\gamma,\theta]} c_r^{[\gamma]} c_s^{[\theta]} - m_{r,s}^{[\gamma,\theta]} p_{r,s}^{[\gamma,\theta]} \quad (3.9)$$

In constructing these equations, we have assumed the existence of a unique equilibrium solution for the $c_r^{[\gamma]}$'s, written $\bar{c}_r^{[\gamma]}$. Provided this exists, an equilibrium solution for the poisoned duplices $p_{r,s}^{[\gamma,\theta]}$ can be found trivially from eq 3.9

$$\bar{p}_{r,s}^{[\gamma,\theta]} = \frac{k_{r,s}^{[\gamma,\theta]} \bar{c}_r^{[\gamma]} \bar{c}_s^{[\theta]}}{m_{r,s}^{[\gamma,\theta]}} \quad (3.10)$$

The other main assumption we have made here is that all four types of monomer are held at a constant concentration, the so-called pool chemical approximation familiar in chemical kinetics.

In the remainder of this paper, we shall ignore poisoning altogether; this can be thought of as equivalent to setting all the coefficients $k_{r,s}^{[\gamma,\theta]}$, $m_{r,s}^{[\gamma,\theta]}$ equal to zero. As written above, these models are extremely complex and indeed represent problems that are of NP type in that their algorithmic "size" is an exponential function of the chain length; this length may itself become unbounded as the reactions proceed. Such intractability means that any analysis must be performed on drastic simplifications of these kinetic equations.

Even these kinetic equations are of course idealised and no experimental systems have yet been identified which exactly realise the steps we are proposing. For example, such polymerizations normally need activating ester linkages, and the polycondensation of mononucleotides diluted in water yields water as a byproduct, thus reducing the concentration of all species. However it is quite feasible that other, more appropriate chemistries will be discovered in the not too distant future.

We shall now analyze two simplified models, one of which exhibits only autocatalysis on the microscopic scale, while the other is fully crosscatalytic.

To simplify the models we shall assume that there is a significant autocatalytic mechanism (specifically we assume that the concentration of a chain $c_r^{[\gamma]}$ is approximately equal to that of its complement $c_r^{[\gamma^*]}$), that all chains have the *same* small crosscatalytic effect, and that the role played by the replicase ribozymes is both crosscatalytic and autocatalytic. Thus we shall replace the vast number of possible but generally unknown rate coefficients in eqs 3.6−3.7 by just four rate constants ϵ, α, χ, and ζ, an approximation which simplifies the flux functions occurring in those equations to

$$J_r^{[\gamma,N_i]} = \left(c_r^{[\gamma]} c_1^{[N_i]} - \frac{\bar{c}_r^{[\gamma]} \bar{c}_1^{[N_i]}}{\bar{c}_{r+1}^{[N_i+\gamma]}} c_{r+1}^{[N_i+\gamma]}\right)(\epsilon + \alpha c_{r+1}^{[\gamma+N_i]} + $$
$$\chi \sum_\theta c_s^{[\theta]} + \zeta c_{r+1}^{[\gamma+N_i]} \sum_\theta c_s^{[\theta]}) \quad (3.11)$$

3.1. Analysis of RNA Formation for the Case of Pure Autocatalysis.

Next we neglect the breakup of chains, so that eqs 3.1 and 3.2 are treated as irreversible; we also ignore the replicase mechanism and template crosscatalysis (that is, we neglect error-prone template synthesis). The resulting equations are made manageable if we analyze a case of this model in which chains catalyse only their own production; thus, a chain

$c_{r+1}^{\gamma+N_3}$ can be constructed in two ways:

$$C_r^\gamma + C_1^{N_3} \xrightarrow{\epsilon} C_{r+1}^{\gamma+N_3}, \quad \text{and} \quad C_r^\gamma + C_1^{N_3} + C_{r+1}^{\gamma+N_3} \xrightarrow{\alpha} 2C_{r+1}^{\gamma+N_3}$$
(3.12)

where α and ϵ denote rate coefficients. By generalizing α to $\alpha + \beta r$ (r being the length of the chain), we can consider a system where longer chains can have greater catalytic effect than short chains. The fluxes are then defined by

$$J_r^{\gamma,N_i} = c_r^\gamma c_1^{N_i} (\epsilon + (\alpha + \beta r) c_{r+1}^{\gamma+N_i}),$$
$$J_r^{N_i,\gamma} = c_r^\gamma c_1^{N_i} (\epsilon + (\alpha + \beta r) c_{r+1}^{N_i+\gamma})$$
(3.13)

with kinetics determined by

$$\dot{c}_r^\gamma = J_{r-1}^{\gamma_1,-\gamma_1+\gamma} + J_{r-1}^{\gamma-\gamma_r,\gamma_r} - \sum_{i=1}^{4} J_r^{\gamma,N_i} - \sum_{i=1}^{4} J_r^{N_i,\gamma} \quad (3.14)$$

We study the system of equations in the pool chemical approximation by assuming a constant concentration of monomers, so that the total matter density will *not* be preserved. We now proceed to solve the Becker–Döring equations and show that growth of long chains can indeed occur.

We seek a solution assuming that the rate coefficients are of magnitudes $\epsilon \ll 1$, $\alpha, \beta = \mathcal{O}(1)$ (that is, the uncatalysed rate is much smaller than the template catalysed rate) and with concentrations depending only on chain length (r). From eqs 3.13 and 3.14, we find that

$$\dot{c}_r = -8 c_r c_1 (\epsilon + (\alpha + \beta r) c_{r+1}) + 2 c_1 c_{r-1} (\epsilon + (\alpha + \beta(r-1)) c_r) \quad (3.15)$$

Strictly speaking, since the equations model irreversible chain growth, the system cannot be described as having an equilibrium solution in the thermodynamic sense; instead, it approaches a steady state solution. In the case $\epsilon \to 0$, this is

$$c_r = \frac{2^{1-r} \bar{c}_1 \, \Gamma(^1/_2(r + \alpha/\beta)) \, \Gamma(^1/_2(2 + \alpha/\beta))}{\Gamma(^1/_2(r+1 + \alpha/\beta)) \, \Gamma(^1/_2(1 + \alpha/\beta))} \quad (3.16)$$

Increasing the catalytic effect of longer chains produces a more rapidly decaying distribution of long chains. This is perhaps slightly counterintuitive: we have made it easier to make long chains and yet find fewer of them; the apparent paradox is resolved by noting that long chains are less likely to 'hang around' as they are busy forming even longer chains. In more realistic models there would be fewer long chains due to the larger errors encountered in replicating long chains and the effect of hydrolysis.

3.2. Analysis of Error-Prone RNA Formation. The above analysis showed, using a very simple model, that autocatalysis alone could produce a population of long chains. We now turn to crosscatalysis to see if this also aids the production of longer chains. Here we are obliged to analyze a more complex model, where all chains act as catalysts for growth. In what follows, we shall analyze the simplest case, for which the rate constants are independent of chain length; in Appendix A we analyze a more complex case where the rate constants are proportional to chain length (and for which more singular behavior is found).

In this case the rate coefficients are given by $\alpha_{r,s}^{\gamma,\theta} = 1$, $\alpha_{r,0}^{\gamma,\theta} = \epsilon$, so that the fluxes can be written as

$$J_r^{N_i,\gamma} = J_r^{\gamma,N_i} = c_r^\gamma c_1^{N_i} \left(\epsilon + \sum_\theta c_s^\theta\right) \quad (3.17)$$

in place of eq 3.13. We continue to use eq 3.14 to relate these fluxes to the rate of change of concentration. We shall use $f_r = \sum_{\gamma:|\gamma|=r} c_r^\gamma$, where the notation $|\gamma|$ means the length of the chain γ. The total monomer concentration of the monomer species is denoted $f_1 = \sum_{i=1}^{4} c_1^{N_i}$ and is held fixed to the same constant for each of the four types of base ($c_1^{N_i} = {}^1/_4 f_1 \, \forall i$).

The form of fluxes (eq 3.17) implicitly assumes that all the rate coefficients are independent of γ, N_i, θ; thus, we look for solutions which only depend on the length of the chain and are independent of its exact composition. We then obtain the simpler equation

$$\dot{f}_r = 2 f_1 f_{r-1} \left(\epsilon + \sum_{s=1}^{\infty} f_s\right) - 2 f_1 f_r \left(\epsilon + \sum_{s=1}^{\infty} f_s\right) \quad (3.18)$$

To solve this type of equation, we define the *generating function*

$$F(z, t) = \sum_{r=1}^{\infty} f_r(t) \, e^{-rz} \quad (3.19)$$

which, from eq 3.18, satisfies the partial differential equation

$$\frac{\partial F(z, t)}{\partial t} = 2 f_1 [\epsilon + F(0, t)] [f_1 e^{-z} - (1 - e^{-z}) F(z, t)] \quad (3.20)$$

To solve eq 3.20 we first find $F(0, t)$ by letting $z \to 0$ in eq 3.20 to give $d/dt \, F(0, t) = 2 f_1^2 [\epsilon + F(0, t)]$ whose solution is

$$F(0, t) = (f_1 + \epsilon) e^{2 f_1^2 t} - \epsilon \quad (3.21)$$

Now that $F(0, t)$ is known, the differential eq 3.20 can be solved to yield

$$F(z, t) = \left(\frac{f_1 e^{-z}}{1 - e^{-z}}\right) [1 - \exp\{-z - (1 - e^{-z})(1 + \epsilon/f_1) (e^{2 f_1^2 t} - 1)\}] \quad (3.22)$$

Ideally we would proceed to find $f_r(t)$ from this by forming the Taylor series in e^{-z}, but it turns out that there is no simple formula for the rth derivative. Instead we interpret the chain length distribution function $f_r(t)/F(0, t)$ as a time-dependent probability density function; it is then straightforward to find how the expected chain length and standard deviation vary with time. Firstly, the total number of chains $\mathbf{N}(t) = F(0, t)$ and so is given by eq 3.21. The total number of chains increases exponentially starting from f_1 at $t = 0$. The expected length of chains grows with the same exponent:

$$\mathbf{E}(t) = \frac{\langle r \rangle}{\mathbf{N}(t)} = \frac{-1}{F(0, t)} \frac{\partial F}{\partial z}\bigg|_{z=0} =$$
$$\frac{(f_1 + \epsilon) e^{4 t f_1^2} + 2(f_1^2 - \epsilon^2) e^{2 t f_1^2} - f_1^2 - 2 f_1 \epsilon + \epsilon^2}{2 f_1((f_1 + \epsilon) e^{2 t f_1^2} - \epsilon)} \quad (3.23)$$

Finally the standard deviation ($\sigma = \sqrt{\mathbf{V}}$) starts from zero and also grows with the same exponent, so that in the large-time

limit the standard deviation has the same order of magnitude as the mean chain length:

$$\mathbf{V}(t) = \frac{\langle r^2 \rangle}{\mathbf{N}} - \frac{\langle r \rangle^2}{\mathbf{N}^2} = \frac{1}{F(0, t)} \frac{\partial^2 F}{\partial z^2}\bigg|_{z=0} - \left(\frac{-1}{F(0, t)} \frac{\partial F}{\partial z}\bigg|_{z=0}\right)^2 \sim$$
$$\frac{1}{12} (e^{2tf_1^2} - 1)(e^{2tf_1^2} + 7 + e^{-2tf_1^2} + 3e^{-4tf_1^2}) + \mathcal{O}(\epsilon) \quad (3.24)$$

None of these quantities blows up in finite time, but they all tend to infinity as $t \rightarrow \infty$. The standard deviation $(\sqrt{\mathbf{V}(t)})$ and the mean $(E(t))$ both tend to infinity exponentially with the same exponent. Thus, after a large time there will be a wide variety of chain lengths present in the system.

3.3. Discussion. The model analyzed in this section allows for the growth of long chains. We have shown that these kinds of Becker−Döring-based models can exhibit a wide variety of behaviors, from the purely autocatalytic model of section 3.1 which approaches a steady-state solution, through the steady slow growth in length and number of chains which occurs in the purely crosscatalytic template-based growth model of section 3.2, to the singular behavior described in Appendix A for an alternative crosscatalytic template-based growth model where rate constants are proportional to chain length. In this last case, the number of chains and expected chain length both blow up in finite time. This behavior would be moderated in the presence of hydrolysis (see sections 5 and 6).

In the next section, we describe a way of analysing the behavior of replicating RNA systems which allows different chain compositions of equal length to have varying concentrations. This will enable us to investigate whether some species (that is, RNA sequences) dominate over others and the possibility that certain species may become extinct.

4. Coarse-Grained Reductions of Microscopic Models

In the previous section we have considered two massive simplifications to the models proposed in section 3 neither of which was capable of describing the effects of the enzymatic reaction mechanism. So far, all the solutions we have managed to find have been for situations where all chains of the same length have the same concentration regardless of their composition. These solutions have shown the dramatic effects, both qualitative and quantitative, which template-based and ribozymic catalysis can have on the kinetics of growth.

In this section we will perform an alternative simplification which enables us to construct solutions which allow different chain compositions to have different concentrations. The main difference between the analysis of the previous two sections and this one is that here we will initially be returning to reversible reactions (although later we shall again impose irreversibility). We thus return to eqs 3.6−3.8, although as noted earlier we shall ignore the inhibition steps.

4.1. Contraction. The equations we start with are eqs 3.6−3.8. Our aim is to perform a coarse-grained rescaling to reduce the number of equations in the system in the same way as has been performed in micelle formation,[21] vesicle formation,[22] and generalized nucleation theory.[23] The same procedure applied here to eqs 3.6−3.8 yields

$$\dot{x}_r^{[\gamma]} = L_{r-1}^{[\gamma - \gamma_{r-\lambda}^r, \gamma_{r-\lambda}^r]} + L_{r-1}^{[\gamma_1^\lambda, -\gamma_1^\lambda + \gamma]} - \sum_{\omega: |\omega| = \lambda} (L_r^{[\gamma, \omega]} + L_r^{[\omega, \gamma]})$$
$$(4.1)$$

$$L_r^{[\gamma,\omega]} = \left(x_r^{[\gamma]} \bar{x}_1^\lambda - \frac{Q_{\Lambda_r}^{[\gamma]}}{Q_{\Lambda_{r+1}}^{[\gamma+\omega]}} x_{r+1}^{[\gamma+\omega]}\right) (\tilde{\epsilon}_r^{[\gamma,\omega]} + \tilde{\alpha}_{r+1}^{[\gamma,\omega]} x_{r+1}^{[\gamma+\omega]} +$$
$$\sum_\theta \tilde{\chi}_{r,s}^{[\gamma,\omega,\theta]} x_s^{[\theta]} + \sum_\theta \sum_{\substack{\xi: \\ \gamma + \omega \subset \xi}} \tilde{\zeta}_{r,s,k}^{[\gamma,\omega,\theta,\xi]} x_s^{[\theta]} x_{k+r}^{[\xi]})^\lambda \quad (4.2)$$

$$L_r^{[\omega,\gamma]} = \left(x_r^{[\gamma]} \bar{x}_1^\lambda - \frac{Q_{\Lambda_r}^{[\gamma]}}{Q_{\Lambda_{r+1}}^{[\omega+\gamma]}} x_{r+1}^{[\omega+\gamma]}\right) (\tilde{\epsilon}_r^{[\omega,\gamma]} + \tilde{\alpha}_{r+1}^{[\omega,\gamma]} x_{r+1}^{[\omega+\gamma]} +$$
$$\sum_\theta \tilde{\chi}_{r,s}^{[\omega,\gamma,\theta]} x_s^{[\theta]} + \sum_\theta \sum_{\substack{\xi: \\ \omega + \gamma \subset \xi}} \tilde{\zeta}_{r,s,k}^{[\omega,\gamma,\theta,\xi]} x_s^{[\theta]} x_{k+r}^{[\xi]})^\lambda \quad (4.3)$$

Note that the Becker-Döring structure[26] present in the original system of rate equations is not destroyed by this approximation.[21−23] Here ω is an arbitrary sequence of length λ, γ_1^λ represents the sequence of the first λ nucleotide monomers of γ, and $\gamma_{r-\lambda}^r$ the last λ.

It is inevitable that this coarse-grained contraction procedure hides some of the subtleties of notation; for example, the term x_1^λ in the contracted flux does not determine the order in which the nucleotides were added−there is no longer a unique form for γ. Thus, the contraction procedure inherently performs a limited averaging in γ-space (sequence space).

As an a posteriori check that these procedures are sensible, we average this system over all possible chain compositions (a "γ-average" of the system (eqs 4.1−4.3). This gives exactly the same results as if we had taken a γ-average of the original system (eqs 3.6−3.8) and then performed the contraction procedure: *the processes of γ-averaging and coarse-graining are commutative*. Both end up yielding

$$\dot{x}_r = 2L_{r-1} - 2(4^\lambda)L_r,$$
$$L_r = \left(x_r x_1^\lambda - \frac{Q_{\Lambda_r}}{Q_{\Lambda_{r+1}}} x_{r+1}\right) (\tilde{\epsilon}_r + \tilde{\alpha}_{r+1} x_{r+1} + \sum_{s=1}^\infty \tilde{\chi}_{r,s} x_s +$$
$$\sum_{s=1}^\infty \sum_{k=1}^\infty \tilde{\zeta}_{r,s,k} x_s x_{r+k})^\lambda \quad (4.4)$$

Below we shall flesh out in more detail two of the simplest approximate schemes which this method produces. To aid the clarity of the resulting models we shall introduce some new notation and drop the tildes from the rate constants, on the understanding that they have been rescaled in the contraction procedure.

4.2. Maximal Contraction of the Rate Processes. In this section we take the contraction procedure described in the previous section as far as possible. We take a value of λ large enough so that there are no chains of length 2λ. Thus we consider only two types of sequence: the monomer forms x_1 and long chains with composition γ, $x_2^{[\gamma]}$.

Consider a combination of crosscatalysis and auto-catalysis (that is template-based chain synthesis with errors), enzymatic replication, as well as the normal reaction mechanism; then there are four distinct ways to make a chain, described in Table 1.

To simplify the notation, since we are only dealing with one length of chain ($|\gamma| = \Lambda = \lambda + 1$), we shall number the types of chain; that is, use Y_n in place of X_2^γ and y_n in place of $x_2^{[\gamma]}$ for the corresponding concentrations. The equation for the rate of

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TABLE 1. Four Mechanisms by which Long chains are Formed

$\Lambda X_1 \rightleftharpoons X_2^\gamma$	uncatalyzed, with forward-rate coefficient = ϵ
$\Lambda X_1 + X_2^\gamma \rightleftharpoons 2X_2^\gamma$	autocatalysis, with forward-rate coefficient = α
$\Lambda X_1 + X_2^\theta \rightleftharpoons X_2^\gamma + X_2^\theta$	crosscatalysis, with forward-rate coefficient = χ
$\Lambda X_1 + X_2^\gamma + X_2^\theta \rightleftharpoons$ $2X_2^\gamma + X_2^\theta$	enzymatic catalytic, with forward-rate coefficient = ζ

change of concentration y_n is then

$$\dot{y}_n = 2L = 2\,(x_1^\Lambda - \beta y_n)\,(\epsilon + \alpha y_n + \chi \sum_{p=1}^{N} y_p + \zeta y_n \sum_{p=1}^{N} y_p)^\lambda \tag{4.5}$$

where $N = 4^\Lambda$ is the number of different chains (β-D-ribonucleotide sequences) of length Λ. The parameter β determines the ratio of chains to monomers at equilibrium. The mechanisms listed in Table 1 are assumed to leave this ratio unchanged; the rates ϵ, α, χ, and ζ only alter the time scale over which the system reaches equilibrium. The backward rates for the mechanisms listed in Table 1 are thus $\beta\epsilon$, $\beta\alpha$, $\beta\chi$, and $\beta\zeta$, respectively.

The advantage of this model equation is that it can be solved in a number of different cases, and it is possible to find how an initially uneven distribution of ribonucleotide chains evolves in time, for instance, whether certain chains die out, or if error-prone template synthesis (crosscatalysis) will even out the differences. The disadvantage is that the model does not contain any information about whether chains will tend to grow longer or how expected chain length varies with time.

4.3. Analysis of Maximally Contracted Model. There are two cases where we shall perform some simple analysis to show that the reduced model derived above generates meaningful results. We shall consider a uniformly growing solution, where all types of chain are present at the same concentration levels, and show that the concentrations increase. We shall then show that this solution is unstable to perturbations which favor the growth of one species over another. The two cases we consider are first constant monomer concentration and second constant density when the backward rates can be neglected ($\beta = 0$).

4.3.1. Prebiotic Soup with Constant Concentration of Ribonucleotide Monomers. We first consider a prebiotic soup which is provided with a constant supply of β-D-ribonucleotide monomers. For simplicity we shall specify the monomer concentration $\bar{x}_1$ in such a way that $2\bar{x}_1^\Lambda = 1$. If we then seek a solution of the form $y_n(t) = Y(t)$, independent of n, we obtain

$$\dot{Y} = (\epsilon + \alpha Y + \chi NY + \zeta NY^2)^\lambda \tag{4.6}$$

In order to solve this with the initial conditions $Y(0) = 0$, we make use of asymptotics with $\alpha, \zeta, \chi \sim \mathcal{O}(1) \gg \epsilon$. The reaction starts on a time scale of $t_1 = \epsilon^{\lambda-1}t$, where $Y = \epsilon Y_1$ satisfies $Y_1' = (1 + \alpha Y_1 + N\chi Y_1)^\lambda$, hence

$$Y = \frac{\epsilon}{(\alpha + N\chi)}\left(\frac{1}{(1 - t/t_c)^{1/(\lambda-1)}} - 1\right) \quad \text{where}$$

$$t_c = \frac{\epsilon^{1-\lambda}}{(\lambda - 1)(\alpha + N\chi)} \tag{4.7}$$

This solution is valid until Y reaches $\mathcal{O}(1)$, when the enzymatic reaction mechanism becomes significant and causes a slightly more rapid blow up.

In practise, however, we do not expect to find all species of RNA chain present in equal quantities. Hence in the real world

this uniform solution must be unstable. We shall now show that it is also unstable in our model and, hence, that our model does indeed predict the preferential growth of one chain type over the others.

To analyze the stability of the uniform solution, we introduce a perturbation around it. Substituting $y_n = Y(1 + \hat{y}_n)$ into eq 4.5 and linearizing we find

$$Y\dot{\hat{y}}_n = \lambda Y\,(\alpha\hat{y}_n + N\zeta Y\hat{y}_n + \chi\hat{\sigma} + \zeta Y\hat{\sigma})\,(\epsilon + \alpha Y + N\chi Y + \zeta NY^2)^{\lambda-1} - \hat{y}_n\,(\epsilon + \alpha Y + N\chi Y + \zeta NY^2)^\lambda \tag{4.8}$$

where $\hat{\sigma} = \sum_{n=1}^{N} \hat{y}_n$. Summing the above equation over the index n, it is possible to show that if $\hat{\sigma}(t_0) = 0$ then $\hat{\sigma}(t) = 0$ for all t. This enables the above equation to be simplified to

$$\dot{\hat{y}}_n = \frac{\hat{y}_n B^{\lambda-1}}{Y}\,[\lambda Y(\alpha + \zeta NY) - B] \tag{4.9}$$

where $B = \epsilon + \alpha Y + \chi NY + \zeta NY^2$. Thus the uniform solution is unstable whenever the difference in the square brackets is positive. For small times the uniform solution is stable, but at larger times it becomes unstable. If $\alpha < N\chi/(\lambda - 1)$ then the instability does not set in until $t \sim t_c$, but if $\alpha > N\chi/(\lambda - 1)$ then the instability occurs for times after

$$t_{\text{instab}} = t_c \left\{ 1 - \left[1 - \left(\frac{\alpha + N\chi}{\alpha\lambda}\right)\right]^{\lambda-1} \right\} \tag{4.10}$$

that is, once the uniform solution has reached the size $Y = Y_{\text{instab}} = \epsilon/[\alpha(\lambda - 1) - N\chi]]$. So we see that the instability occurs when the ratio of autocatalysis to crosscatalysis (equivalently, the ratio of error-free to error-prone template synthesis) exceeds a certain threshold. Put another way, *for certain species (sequences) to grow while others become extinct, the template-based copying of RNA needs to achieve a certain specified level of accuracy.*

4.3.2. Prebiotic Soup with Constant Total Mass of Ribonucleotide Bases. In the case of a prebiotic soup with a constant total quantity of ribonucleotide bases (another way of putting this is to say that the total nucleotide mass density, which may be present in monomers or chains, is constant), we eliminate the monomer concentration to leave

$$\dot{y}_n = 2\,(\rho - \Lambda \sum_{n=1}^{N} y_n)^\Lambda(\epsilon + \alpha y_n + \chi \sum_{p=1}^{N} y_p + \zeta y_n \sum_{p=1}^{N} y_p)^\lambda \tag{4.11}$$

To find the uniform solution and determine its stability, we once again use the substitution $y_n = Y(1 + \hat{y}_n)$. Inserted into the above, this yields

$$\dot{Y} = 2(\rho - \Lambda NY)^\Lambda(\epsilon + \alpha Y + \chi NY + \zeta NY^2)^\lambda \tag{4.12}$$

$$\dot{\hat{y}}_n = \frac{2\hat{y}_n}{Y}\,(\rho - \Lambda NY)^\Lambda(\epsilon + \alpha Y + \chi NY + \zeta NY^2)^{\lambda-1}\,[\lambda(\alpha Y + \zeta Y^2 N) - (\epsilon + \alpha Y + N\chi Y + N\zeta Y^2)] \tag{4.13}$$

where we have already made use of the fact that $\hat{\sigma} = \sum_{n=1}^{N} \hat{y}_n = 0$ for all time. The leading order solution to the former (eq 4.12) is similar to the uniform solution in the constant monomer case above:

$$Y = \frac{\epsilon}{(\alpha + N\chi)} \left(\frac{1}{(1 - t/t_c)^{1/(\lambda-1)}} - 1 \right),$$

$$\text{where} \quad t_c = \frac{\epsilon^{1-\lambda}}{2\rho^\Lambda(\lambda - 1)(\alpha + N\chi)} \quad (4.14)$$

The uniform solution is then unstable for $t > t_{\text{instab}}$, where t_{instab} is given by eq 4.10 but with t_c determined by eq 4.14 rather than eq 4.7.

4.4.3. Remarks. It is possible to prove similar results for the case in which the backward rates are not neglected ($\beta \neq 0$), since all the analysis presented in the above section relies on a solution for which Y is small ($Y \sim \mathcal{O}(\epsilon)$). The solution remains valid until $Y \sim \mathcal{O}(1)$. Since all the instabilities found above arise while Y is still $\mathcal{O}(\epsilon)$, they will also arise in systems with $\beta \neq 0$, because in such systems the uniform solution and its stability criteria will not be altered by the β term until Y reaches $\mathcal{O}(1)$.

A more general notion of stability that we have not addressed is that for which the rate coefficients differ for different ribonucleotide chain sequences. By this we mean, for example, that different chains y_n will have different autocatalytic rate constants, α_n. A more detailed analysis could be carried out, and similar results to those presented above would be produced. The main difference is in the selection of which chains proliferate. In the case studied here, this is determined by initial conditions, but for cases in which the autocatalytic rate varies with chain composition, this provides another selection mechanism and those chains with the largest autocatalytic rates will grow at the expense of the other species. Thus perturbations to the autocatalytic rates accentuate the instability analyzed above.

4.4. Almost Maximal Contraction. In the foregoing work, we have used a maximally-contracted Becker–Döring model to analyze the kinetics of formation of RNA chains. In order to derive a slightly more accurate model, we can use the same theory as described in section 4.1, but instead of using a coarse-graining mesh so large that only monomers and chains are captured, we use a finer-grained mesh which includes two types of chains: short ones (of length Λ) and long ones (of length $\Lambda + \mu$). Formally, the kinetic equations are then

$$\dot{x}_2^{[\gamma]} = L_1^{[\gamma^1, \gamma_2^\Lambda]} + L_1^{[\gamma^\lambda, \gamma_\Lambda^\Lambda]} - \sum_{\omega:|\omega|=\mu}(L_2^{[\gamma,\omega]} + L_2^{[\omega,\gamma]})$$

$$\dot{x}_3^{[\gamma]} = L_2^{[\gamma^\Lambda, \gamma_{\Lambda+\mu}^{\Lambda+\mu}]} + L_2^{[\gamma^\mu, \gamma_{\mu+1}^{\Lambda+\mu}]} \quad (4.15)$$

$$L_1^{[N,\gamma]} = (x_1^\Lambda - \beta_2 x_2^{[N+\gamma]})(\epsilon + \alpha_2 x_2^{[N+\gamma]} + \chi \sum_\theta x_2^{[\theta]} +$$

$$\chi \sum_\theta x_3^{[\theta]} + x_2^{[N+\gamma]} \sum_\theta \zeta x_2^{[\theta]} + x_2^{[N+\gamma]} \sum_\theta \zeta x_3^{[\theta]} +$$

$$\sum_{\theta,\omega} \zeta x_3^{[N+\gamma+\omega]} x_2^{[\theta]} + \sum_{\theta,\omega} \zeta x_3^{[N+\gamma+\omega]} x_3^{[\theta]} +$$

$$\sum_{\theta,\omega} \zeta x_3^{[\omega+N+\gamma]} x_2^{[\theta]} + \sum_{\theta,\omega} \zeta x_3^{[\omega+N+\gamma]} x_3^{[\theta]})^\lambda$$

$$L_2^{[\gamma,\omega]} = (x_2^{[\gamma]} x_1^\mu - \beta_3 x_3^{[\gamma+\omega]})(\epsilon + \alpha x_3^{[\gamma+\omega]} + \chi \sum_\theta x_3^{[\theta]} +$$

$$\chi \sum_\theta x_3^{[\theta]} + \zeta x_3^{[\gamma+\omega]} \sum_\theta x_2^{[\theta]} + \zeta x_3^{[\gamma+\omega]} \sum_\theta x_3^{[\theta]})^\mu$$

This system has mass density given by

$$\rho = 4x_1 + \sum_{\gamma:|\gamma|=\Lambda} \Lambda x_2^{[\gamma]} + \sum_{\gamma:|\gamma|=\Lambda+\mu} (\Lambda + \mu)x_3^{[\gamma]} \quad (4.16)$$

Such a complex system is more easily understood if we generalize the notation of the previous section. In the special cases where $\mu = \lambda + 1$, which we shall study in more detail later, we simplify the notation for short chains $x_2^{[\gamma]}$ to y_n, where n encapsulates the information stored in γ, and we also define y_{mn} to be the concentration of a chain $y_{m,n}$. Then we can write

$$\dot{y}_n = (x_1^\Lambda - \beta_2 y_n)(\epsilon + \alpha y_n + \sum_{p=1}^{N} \chi y_p + \sum_{p,q=1}^{N,N} \chi y_{p,q} +$$

$$\sum_{p=1}^{N} \zeta y_n y_p + \sum_{p,q=1}^{N,N} \zeta y_n y_{p,q} + \sum_{m,p=1}^{N,N} \zeta y_{m,n} y_p + \sum_{m,p,q=1}^{N,N,N} \zeta y_{m,n} y_{pq,} +$$

$$\sum_{m,p=1}^{N,N} \zeta y_{n,m} y_p + \sum_{m,p,q=1}^{N,N,N} \zeta y_{n,m} y_{p,q})^\lambda \quad (4.17)$$

$$-\sum_{r=1}^{N}(y_r x_1^\Lambda - \beta_3 y_{n,r})(\epsilon + \alpha y_{n,r} + \chi \sum_{p=1}^{N} y_p + \chi \sum_{p,q=1}^{N} y_{p,q} +$$

$$\zeta y_{n,r} \sum_{p=1}^{N} y_p + \zeta y_{n,r} \sum_{p,q=1}^{N,N} y_{p,q})^\Lambda$$

$$-\sum_{r=1}^{N}(y_r x_1^\Lambda - \beta_3 y_{r,n})(\epsilon + \alpha y_{r,n} + \chi \sum_{p=1}^{N} y_p + \chi \sum_{p,q=1}^{N,N} y_{p,q} +$$

$$\zeta y_{r,n} \sum_{p=1}^{N} y_p + \zeta y_{r,n} \sum_{p,q=1}^{N,N} y_{p,q})^\Lambda$$

$$\dot{y}_{m,n} = (y_m x_1^\Lambda + y_n x_1^\Lambda - 2\beta_3 y_{m,n})(\epsilon + \alpha y_{m,n} + \chi \sum_{p=1}^{N} y_p +$$

$$\chi \sum_{p,q=1}^{N,N} y_{p,q} + \zeta y_{m,n} \sum_{p=1}^{N} y_p + \zeta y_{m,n} \sum_{p,q=1}^{N,N} y_{p,q})^\Lambda \quad (4.18)$$

This system of ordinary differential equations can be most readily understood in terms of the set of macroscopic chemical reactions comprising it. Let us use X_1 to denote the monomer species, Y_n to denote a short chain of type n, and $Y_{m,n}$ to denote a long chain whose composition is identical to that of a short chain of type m joined to a short chain of type n. The reactions which the above kinetic scheme describes are listed in Table 2. It will be recalled that, owing to the coarse-graining scheme applied, the quantities Y_n and $Y_{m,n}$ represent "averages" over sets of chain lengths and sequences.

The constant quoted after each step in the reaction mechanism is the rate coefficient associated with that process. We shall not analyze this still very complex model here, however, as its complexity would be compounded by the subsequent introduction of hydrolytic fragmentation processes. Instead, the above model will be simplified further, and used in the following sections which include hydrolysis for the first time.

To conclude this section, we have shown that a systematic coarse-grained contraction procedure can be employed to greatly reduce the number of rate equations and different intermediates that need to be considered. Such a procedure yields equations which are amenable to theoretical as well as numerical analysis and we shall show in section 5 how the most drastic approximation—the maximally contracted system--can still account for the way in which a combination of template-based synthesis and ribozymic replicase activity amplifies initial differences in RNA chain concentrations. In section 6, we shall return to the "almost maximally contracted system" when

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TABLE 2. Reactions Described in the Kinetic Equations 4.17 and 4.18, Together with Their Forward Rate Constants and a Brief Description of Each

$\Lambda X_1 \rightarrow Y_n$	ϵ	uncatalyzed formation of short chains
$Y_n + \Lambda X_1 \rightarrow Y_{n,m}$	ϵ	uncatalyzed formation of long chains
$Y_n + \Lambda X_1 \rightarrow 2Y_n$	α	autocatalysis of short chains
$Y_{m,n} + Y_m + \Lambda X_1 \rightarrow 2Y_{m,n}$	α	autocatalysis of long chains
$Y_m + \Lambda X_1 \rightarrow Y_m + Y_n$	χ	crosscatalysis of short by short
$Y_{m,n} + Y_k + \Lambda X_1 \rightarrow Y_{m,n} + Y_{k,l}$	χ	crosscatalysis of long by long
$Y_{m,n} + \Lambda X_1 \rightarrow Y_{m,n} + Y_k$	χ	crosscatalysis of short by long
$Y_m + Y_k + \Lambda X_1 \rightarrow Y_m + Y_{k,l}$	χ	crosscatalysis of long by short
$Y_p + Y_n + \Lambda X_1 \rightarrow 2Y_n + Y_p$	ζ	ribozymic synthesis of short chains
$Y_{p,q} + Y_n + \Lambda X_1 \rightarrow 2Y_n + Y_{p,q}$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{m,n} + \Lambda X_1 \rightarrow Y_n + Y_{m,n} + Y_p$	ζ	ribozymic synthesis of short chains
$Y_{p,q} + Y_{m,n} + \Lambda X_1 \rightarrow Y_n + Y_{m,n} + Y_{p,q}$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{n,m} + \Lambda X_1 \rightarrow Y_n + Y_{n,m} + Y_p$	ζ	ribozymic synthesis of short chains
$Y_{p,q} + Y_{n,m} + \Lambda X_1 \rightarrow Y_n + Y_{n,m} + Y_{p,q}$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{m,n} + Y_m + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_p$	ζ	ribozymic synthesis of long chains
$Y_{p,q} + Y_{m,n} + Y_m + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_{p,q}$	ζ	ribozymic synthesis of long chains
$Y_p + Y_{m,n} + Y_n + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_p$	ζ	ribozymic synthesis of long chains
$Y_{p,q} + Y_{m,n} + Y_n + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_{p,q}$	ζ	ribozymic synthesis of long chains

incorporating hydrolysis into the theoretical description of RNA chain replication.

5. RNA Formation Including Hydrolysis

From a chemical point of view, a crucial omission from all of the foregoing models is hydrolysis. This is the process by which polymeric ribonucleotide chains are prevented from growing to unlimited lengths. Many of the models analyzed above have the unfortunate property of producing infinitely long chains in a finite time. This implies the presence of mathematical singularities within the kinetic equations and by itself indicates that certain essential physicochemical processes have been overlooked.

So far, our general models of RNA formation have been based on the Becker–Döring theory, whereby chains grow or fragment through *one step* addition or removal of β-D-ribonucleotide monomers. For more general coagulation–fragmentation processes, in which arbitrarily large amounts of polymer may be added or removed, one can use a description based on the general Smoluchowski equations (eq 5.1) instead of the Becker–Döring equations. While the Becker–Döring theory evidently works well for the stepwise growth of RNA chains, hydrolysis can only be accounted for by a general fragmentation term, since the loss of a single monomer from the end of an RNA oligomer is an exceedingly poor approximation to hydrolysis. Therefore, in the models described below we shall retain the original Becker–Döring assumptions when modelling the aggregation mechanism, but we shall include a general Smoluchowski fragmentation term to handle the effects of hydrolysis.

We wish to emphasize that we do not make any particular assumptions about the nature of hydrolysis. We certainly do not seek to describe it in terms of equilibrium processes (which would enable an arbitrary hydrolytic chain fragmentation to be equivalently described in terms of a sequence of stepwise processes, and to which Le Chatelier's principle could be applied). Rather, hydrolysis is modelled explicitly by a distinct kinetic process which splits a chain in two at an arbitrary point and is thus of general Smoluchowski fragmentation form.

In the rest of this section, our model of possibly catalytic Becker–Döring chain growth combined with a general fragmentation term will be analyzed by the use of generating function and coarse-grained reduction techniques.

5.1. Formulation of the Smoluchowski Coagulation–Fragmentation Equations for RNA Formation with Hydrolysis. The full Smoluchowski coagulation–fragmentation equations for the rate of change of concentration of a polymeric

chain of length r are

$$\dot{c}_r = \frac{1}{2}\sum_{k=1}^{r-1} W_{k,r-k} - \sum_{k=1}^{\infty} W_{r,k}, \quad r = 2, 3, \ldots$$

$$W_{r,s} = a_{r,s}c_r c_s - b_{r,s}c_{r+s} \quad (5.1)$$

where the rate coefficients for the aggregation and fragmentation steps $a_{r,s}$ and $b_{r,s}$, respectively, now carry two suffices, indicative of the fact that clusters of sizes r and s can reversibly coalesce to form a cluster of size $(r + s)$. We shall analyze two versions of this system: in one, the monomer concentration c_1 will be assumed constant; in the other, the monomer concentration will be allowed to vary, and the density $\rho = \sum_{r=1}^{\infty} rc_r$ will be held constant.

To recover the original Becker–Döring equations from eq 5.1, we impose the condition that there can be no polymer–polymer aggregation, so that a ribonucleotide chain can only aggregate via one monomer at a time. Thus we impose the following form

$$a_{r,s} = a_r\delta_{s,1} + a_s\delta_{r,1} \quad (5.2)$$

on the forward coefficients $a_{r,s}$ (note that this implies $a_{11} = 2a_1$), where $\delta_{i,j}$ is the Kronecker delta satisfying $\delta_{i,j} = 1$ only if $i = j$ and is zero otherwise.

Just as template-based catalysis was included in the Becker–Döring model of section 3, it can be incorporated within this more general coagulation–fragmentation model. This is achieved by replacing $a_{r,s}$ above by $a_{r,s} + \sum_{k=1}^{\infty} f_{r,s,k}c_k$, where $f_{r,s,k}$ is another set of rate coefficients for the template synthesis of oligomeric β-D-ribonucleotides.

We shall make extensive use of generating function techniques and choose simple forms for the coefficients $a_{r,s}$ and $b_{r,s}$ so that these techniques may be used to their full potential. Initially, in the same manner as in eq 3.19, we define the generating function

$$C(z, t) = \sum_{r=1}^{\infty} c_r(t)e^{-zr} \quad (5.3)$$

which can be viewed as a discrete Laplace transform. This transforms the problem from having one discrete and one continuous independent variable (r, t) to two continuous variables (z, t). Note that putting $z = 0$ in the above expression gives the quantity $C_0(t) = C(0, t)$ which is the total number of

chains in the system. A second important quantity in the analysis of these problems is

$$u(z, t) = -\frac{\partial C}{\partial z} = \sum_{r=1}^{\infty} rc_r e^{-zr} \tag{5.4}$$

and inserting $z = 0$ in this yields the density, $\rho = u_0(t) = u(0, t)$.

In the main, we shall choose constant rate coefficients, although forward rate coefficients of the form $a_r = a + \tilde{a}r$ can be analyzed without much extra difficulty. Two types of backward rate coefficients can also be analyzed: (i) $b_{r,s} = b$, which we shall concentrate on, and (ii) $b_{r,s} = b/(r + s - 1)$ which we do not consider further here. The choice of a constant hydrolysis rate implied by (i) is a reasonable first approximation; the choice (ii), where long chains have a smaller chance of undergoing hydrolysis than short ones, is less tenable.

As was done earlier in the paper, two types of prebiotic scenario are analyzed below. The first has constant mass density ρ, for which a simple closed system of equations can be generated for the monomer concentration ($c_1(t)$) and the total concentration of polymer chains including monomers ($C_0(t)$). These give some indication of the polydispersity of the chains present in the soup since the average RNA chain length is then equal to

$$\mathbf{E}(t) = \langle r \rangle = \frac{\sum rc_r(t)}{\sum c_r(t)} = \frac{\rho}{C_0(t)} \tag{5.5}$$

Typically we are interested in the evolution of the system starting from an initial condition where all the mass is in monomeric form; that is, $c_1(0) = \rho$, $c_r(0) = 0 \ \forall r \geq 2$ which implies $C_0(0) = \rho$. Systems of this type are analyzed in sections 5.2 and 5.3.

In section 5.4, a second prebiotic scenario is analyzed, which has a fixed monomer concentration (c_1), so that the total ribonucleotide density $u_0(t)$ may vary. This is equivalent to the pool chemical approximation. In this case we can form a simple closed system of equations for the density $u_0(t)$ and the total number of polymer chains $C_0(t)$.

In the situations we initially consider, the only type of template-based synthesis analyzed involves error-prone replication or crosscatalysis (in fact, pure autocatalysis cannot be analyzed using generating function methods as the type of nonlinearity which it introduces is not amenable to transform techniques). For this reason we have not given the full formulation of the problem, including details of individual sequences, as that would introduce autocatalysis both directly and as part of the ribozymic replicase polymerization mechanism. However, these more complex mechanisms will be introduced in the next section (section 6) when we consider massively contracted models.

5.2. Constant Monomer Concentrations: Pure Template-Based RNA Synthesis. In this section we shall reconsider the case analyzed in the Appendix which has forward rate constants proportional to chain length; there, the total mass in the system and the average chain length both blew up in finite time. We now show that the incorporation of hydrolysis into the model prevents such unrealistic behavior.

Whereas an RNA chain can grow only by adding a single monomer to one or other end of the chain, a chain can split into two parts at any position along its length. Since the general fragmentation term we have included gives each phosphate bond an equal chance of breaking, and the number of bonds in a chain

is proportional to chain length, the reverse reaction rate coefficient is effectively proportional to chain length. Thus we expect that a general hydrolytic fragmentation mechanism with constant rate coefficients will be able to prevent unbounded chain growth in a polymerization mechanism with forward rate constants proportional to chain length.

The equations which describe pure template synthesis together with hydrolysis in a system with constant ribonucleotide monomer concentration are

$$\dot{c}_r = \sum_{s=1}^{\infty} f(r-1)sc_s c_{r-1}c_1 - \sum_{s=1}^{\infty} frsc_s c_r c_1 - \tfrac{1}{2}b(r-1)c_r +$$
$$\sum_{s=1}^{\infty} bc_{r+s} \tag{5.6}$$

The generating function $C(z, t)$ of eq 5.3 is then determined by

$$\frac{\partial C}{\partial t} = fc_1^2 u_0 e^{-z} - fc_1 u_0 u(1 - e^{-z}) - \tfrac{1}{2} bu + \tfrac{1}{2}bC +$$
$$bc_1 e^{-z} + b\left(\frac{C_0 e^{-2z} - C}{1 - e^{-z}}\right) \tag{5.7}$$

where $u = -\partial C/\partial z$ in turn satisfies

$$\frac{\partial u}{\partial t} = fc_1^2 u_0 e^{-z} + fc_1 u_0 \frac{\partial u}{\partial z}(1 - e^{-z}) + fc_1 u_0 u e^{-z} + \tfrac{1}{2} b\frac{\partial u}{\partial z} +$$
$$\tfrac{1}{2}bu + bc_1 e^{-z} - bC_0 e^{-z} - b\left(\frac{u(1 - e^{-z}) + (C - C_0)e^{-z}}{(1 - e^{-z})^2}\right) \tag{5.8}$$

A closed system can then be formed for the total number of chains $C_0(t)$ and total mass of RNA chains $u_0(t)$ by letting $z \to 0$ in eqs 5.7 and 5.8, giving

$$\dot{C}_0 = fc_1^2 u_0 + \tfrac{1}{2}bu_0 - \tfrac{3}{2}bC_0 + bc_1 \tag{5.9}$$

$$\dot{u}_0 = fc_1 u_0^2 + fc_1^2 u_0 - bC_0 + bc_1 \tag{5.10}$$

In general, explicit solutions of this pair of equations are not available, but special cases can be analyzed, and a fuller picture built up from these.

The system has equilibrium solutions at

$$\bar{u}_0 = \frac{c_1}{6}\left(\frac{b}{fc_1^2} - 1 \pm \sqrt{\frac{b^2}{f^2 c_1^4} - \frac{14b}{fc_1^2} + 1}\right) \tag{5.11}$$

where this is well-defined, namely, for $b/fc_1^2 \geq 7 + \sqrt{48} \approx 13.9$ (for $b/fc_1^2 \leq 7 - \sqrt{48} \approx 0.07$, both roots correspond to negative values of the density $\bar{u}_0$).

5.2.1. The Case $b = 0$. For $b = 0$ the system of two ordinary differential equations is solvable. Making the substitution $u_0 = 1/w$ produces a linear equation which can be solved and leads to

$$u_0(t) = \frac{c_1}{2e^{-fc_1^2 t} - 1} \tag{5.12}$$

thus the system blows up at time $t = t_g = \log(2)/fc_1^2$ which, as is to be expected, is the result obtained in the Appendix, since $b = 0$ corresponds to no hydrolysis. For small b there will be only minor modifications to this behavior.
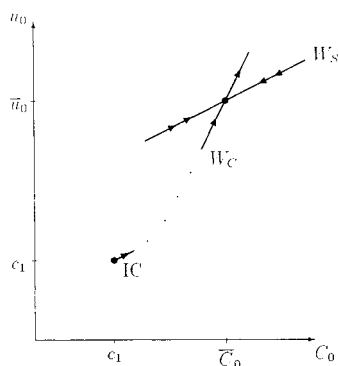
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Figure 1. Diagram of the (C_0, u_0) phase plane for the case $b = b_c$. IC marks the initial condition imposed on the system where all nucleotide matter is in monomeric form and so both the mass $u_0 = c_1$ and the number of objects in the system $C_0 = c_1$. W_C is the center manifold emanating from the equilibrium configuration of the system, along which the system slowly moves. W_S marks the stable manifold of the equilibrium point; its existence shows that the center manifold is itself stable in that points close to the stable manifold are attracted to it. At equilibrium the number of chains $C_0 = \bar{C}_0$ and the mass $u_0 = \bar{u}_0$.

5.2.2. The Case $b = b_c = (7 + 4\sqrt{3})fc_1^2$. At the smallest value of b where an equilibrium solution exists, the equilibrium values of C_0 and u_0 are $^1/_3(1 + 2\sqrt{3})c_1$ and $(1 + 2/\sqrt{3})c_1$, respectively. An expansion about the equilibrium point using

$$C_0 = {}^1/_3 c_1(1 + 2\sqrt{3})(1 + g), \quad u_0 = c_1(1 + {}^2/_3\sqrt{3})(1 + w) \quad (5.13)$$

reveals the existence of one stable and one center manifold.[27] The existence of a center manifold is to be expected since at $b = b_c$ the system undergoes a saddle−node bifurcation:

$$\frac{1}{fc_1^2}\frac{dg}{dt} = \left(\frac{3}{10\sqrt{3} - 17}\right)(-(\sqrt{3} + {}^1/_2)g + (\sqrt{3} - {}^1/_2)w) \quad (5.14)$$

$$\frac{1}{fc_1^2}\frac{dw}{dt} = \left(\frac{2}{2\sqrt{3} - 3}\right)(-(\sqrt{3} + {}^1/_2)g + (\sqrt{3} - {}^1/_2)w + {}^3/_4 w^2) \quad (5.15)$$

The eigenvector corresponding to the stable manifold is $\left(\frac{21/2 + 6\sqrt{3}}{5 + 8/\sqrt{3}}\right)$ and that corresponding to the center manifold is $\left(\frac{\sqrt{3} - 1/2}{\sqrt{3} + 1/2}\right)$. This situation is semistable in that the equilibrium position is only stable from one side. If too much matter is inserted into the system, then the system's behavior is divergent, gaining mass without bound. However, if the system is initiated with only a little matter present, it will evolve smoothly and slowly approach an equilibrium solution. The situation is summarized in Figure 1.

Systems which start below the stable manifold, the lines marked W_S on Figure 1, are attracted to the equilibrium solution along the center manifold (W_C); however, systems initiated above W_S gain mass and are repelled from the equilibrium point. Thus the trajectory we are interested in (that starting from the initial conditions $C(0) = c_1 - u_0(0)$) does approach the equilibrium solution.

5.2.3. The Case $b \gg 1$. For asymptotically large b, the system has a critical point near

$$C_0 \sim c_1\left(1 + \frac{2fc_1^2}{b}\right), \quad u_0 \sim c_1\left(1 + \frac{4fc_1^2}{b}\right) \quad (5.16)$$

The nature of this critical point is a stable node, so the initial conditions at $C_0 = u_0 = c_1$ are attracted to this stable equilibrium

configuration. (There is also a saddle point at $u_0 \sim b/3fc_1$, $C_0 \sim b/9fc_1$.) This is intuitively in line with what we expect: b very large implies that hydrolysis dominates the kinetics, making it almost impossible to build long chains; the few that are built rapidly break up, so that the system remains mainly in monomeric form.

5.2.4. Summary. This example shows that the inclusion of hydrolysis, according to which RNA chains split at any point along their length, provides a powerful mechanism which can prevent chains from growing infinitely long. With a constant hydrolysis rate (independent of chain length) the mechanism prevents infinitely long chains forming in RNA systems in which the growth rate is proportional to chain length.

In section 3.2, a model with coefficients independent of chain length was analyzed and shown to have divergent behavior in the limit $t \to \infty$. In this limit, both the number of chains and mass of material in chains grow without bound in such a way that the expected chain length also grows unboundedly. In the Appendix it is shown that in models where rate constants are proportional to chain length, such a singularity can occur in finite time.

This section has shown that if a significant degree of hydrolysis is included into such models, it prevents this unphysical behavior. If the hydrolysis rate exceeds a threshold value, there is a stable equilibrium configuration to which the system will tend.

5.3. Constant Rate Coefficients and Constant Nucleotide Monomer Concentrations. We now consider a more general model which includes both catalyzed and uncatalyzed RNA chain growth mechanisms. We assume that all reaction rates are independent of chain length, using f to denote the catalyzed rate and a the uncatalyzed rate of addition of nucleotide monomers to a chain.

For $r > 1$, the equation

$$\dot{c}_r = ac_1c_{r-1} - ac_1c_r + fc_1c_{r-1}\sum_{s=1}^{\infty} c_s - fc_1c_r\sum_{s=1}^{\infty} c_s - $$
$$ {}^1/_2 b(r - 1)c_r + b\sum_{s=1}^{\infty} c_{r+s} \quad (5.17)$$

governs the rate of change of concentrations. The generating function then satisfies

$$\frac{\partial C}{\partial t} = ac_1^2 e^{-z} + fc_1^2 C_0 e^{-z} - ac_1C(1 - e^{-z}) - $$
$$fc_1 C_0 C(1 - e^{-z}) + {}^1/_2 bC - {}^1/_2 bu + bc_1 e^{-z} + \frac{b(C_0 e^{-2z} - C)}{(1 - e^{-z})} \quad (5.18)$$

where $u = -\partial C/\partial t$ satisfies the partial differential equation

$$\frac{\partial u}{\partial t} = c_1 e^{-z}(c_1 + C)(a + fC_0) - uc_1(1 - e^{-z})(a + fC_0) + $$
$$ {}^1/_2 bu + {}^1/_2 b\frac{\partial u}{\partial z} + bc_1 e^{-z} - bC_0 e^{-z} - $$
$$\frac{b[(1 - e^{-z})u + e^{-z}(C - C_0)]}{(1 - e^{-z})^2} \quad (5.19)$$

Again, these equations cannot be solved in general, but useful information about their solutions can be obtained by considering the limit $z \to 0$ in which they reduce to a coupled pair of ordinary differential equations:

$$\dot{C}_0 = (fc_1^2 - {}^3/_2b) C_0 + ac_1^2 + bc_1 + {}^1/_2bu_0$$

$$\dot{u}_0 = fc_1C_0^2 + (fc_1^2 + ac_1 - b)C_0 + ac_1^2 + bc_1 \quad (5.20)$$

We seek an equilibrium solution of these equations by first solving the latter quadratic equation to find C_0 and then the former linear expression which gives u_0 in terms of C_0. Let us define

$$B = \frac{b}{ac_1 + fc_1^2}, \quad \chi = \frac{fc_1^2}{ac_1 + fc_1^2} \quad (5.21)$$

so that B is the reciprocal of the equilibrium constant for the overall reaction (the ratio of the total hydrolytic fragmentation rate to the total polymerization rate), while χ is a measure of the effectiveness of catalysis, since it is the ratio of the catalysis rate to the total aggregation rate ($0 < \chi < 1$). Thus the equilibrium value of C_0 is given by

$$\bar{C}_0 = \frac{1}{2\chi}(B - 1 \pm \sqrt{(B-1)^2 - 4\chi}) \quad (5.22)$$

Due to the form of the discriminant, if the roots for $\bar{C}_0$ are real then they have the same sign. If $B < 1$ then they are both negative or both complex and hence are not relevant for the large time asymptotics of the system (eq 5.20). In this case the total number of chains and mass of material must both tend to infinity. If $1 < B < 1 + 2\sqrt{\chi}$, then the roots for $\bar{C}_0$ are complex and hence again irrelevant when considering the large time asymptotics of the system (eq 5.20). However, if $B > 1 + 2\sqrt{\chi}$, then hydrolysis is strong enough to prevent such unphysicochemical divergences, and in the large-time limit the system may be attracted to such an equilibrium configuration.

5.4. Constant Rate Coefficients and Constant Nucleotide Density. In order to consider the constant-density form of the equations, we need to construct a new equation for the monomer concentration which accounts for the loss of monomer as the ribonucleotide chains grow. With constant reaction rate coefficients, the kinetic equations are

$$\dot{c}_r = ac_1c_{r-1} - ac_1c_r + fc_1c_{r-1}\sum_{s=1}^{\infty} c_s - fc_1c_r\sum_{s=1}^{\infty} c_s - $$
$$ {}^1/_2b(r-1)c_r + b\sum_{s=1}^{\infty} c_{r+s} \quad (5.23)$$

$$\dot{c}_1 = -ac_1^2 - ac_1\sum_{s=1}^{\infty} c_s - fc_1^2\sum_{s=1}^{\infty} c_s - fc_1\left(\sum_{s=1}^{\infty} c_s\right)\left(\sum_{s=1}^{\infty} c_s\right) + $$
$$ b\sum_{s=1}^{\infty} c_{s+1} \quad (5.24)$$

which can again be analyzed using generating function techniques; from eq 5.3

$$\frac{\partial C}{\partial t} = c_1 e^{-z}(C - C_0)(a + fC_0) - c_1C(a + fC_0) + be^{-z}C_0 + $$
$$ {}^1/_2bC - {}^1/_2bu + \frac{b(C_0e^{-2z} - C)}{(1 - e^{-z})} \quad (5.25)$$

In this case the equation for $\partial u/\partial t$ is not so important for us since the imposition of constant density implies $\dot{u}_0 = 0$. This leads to the following coupled pair of ordinary differential equations for the total number of chains and the monomer concentration

$$\dot{C}_0 = -fc_1C_0^2 - (ac_1 + {}^1/_2b) C_0 + {}^1/_2 b\rho \quad (5.26)$$

$$\dot{c}_1 = -(a + fC_0)c_1^2 - (aC_0 + fC_0^2 + b)c_1 + bC_0 \quad (5.27)$$

Starting from initial conditions in which all the available ribonucleotide mass is in monomeric form, which implies $c_1(0) = \rho$, $C_0(0) = \rho$, we expect the system to approach an equilibrium configuration with significantly less monomers, and fewer chains, but where the average chain length $\langle r \rangle := \rho/C_0$ is much larger than unity.

It is not possible to explicitly find equilibrium points of this system, but we can show that they exist and occur in the correct region of phase space. First note that $\dot{C}_0 = 0$ implies that at equilibrium

$$\bar{c}_1 = \frac{b(\rho - \bar{C}_0)}{2\bar{C}_0(a + f\bar{C}_0)} \quad (5.28)$$

Then the condition $\dot{c}_1 = 0$ corresponds to finding roots of the function

$$G(\bar{C}_0) := b(\rho - \bar{C}_0)^2 + 2\bar{C}_0(\rho - \bar{C}_0)(f\bar{C}_0^2 + a\bar{C}_0 + b) - $$
$$ 4\bar{C}_0^3(a + f\bar{C}_0) \quad (5.29)$$

From this it is possible to see that $G(0) = b^2\rho > 0$ and $G(\rho) = -4b\rho^3(a + f\rho) < 0$ so there is an equilibrium value of $\bar{C}_0$ between zero and ρ, and from eq 5.28 we can find a corresponding value of $\bar{c}_1$.

In the limit $b \to 0$, it is possible to obtain the equilibrium solution asymptotically; in this case it is

$$\bar{C}_0 = \frac{1}{3}\rho, \quad \bar{c}_1 = 3b/(3a + f\rho) \quad (5.30)$$

so the monomer concentration is small and the equilibrium distribution of chains has mean length $\langle r \rangle = \rho/\bar{C}_0 = 3$.

The ribonucleotide polymer chain distribution can then be found by solving eq 5.25 with $\partial C/\partial t = 0$ and $u = -\partial C/\partial z$ to give

$$\bar{C}(z) = {}^1/_3\rho[1 - {}^3/_2e^z(e^{2z} - 1) + {}^3/_2e^{z+2e^{-z}}(e^z - 1)^2] \quad (5.31)$$

from which we can find the concentration distribution

$$\bar{c}_r = \frac{\rho 2^r (r - 1)}{(r + 1)! (r + 3)} \quad (5.32)$$

This corresponds to a pure aggregation Becker-Döring model where coagulation stops as $c_1 \to 0$. Figure 2 shows this profile which demonstrates how effective even a small amount of hydrolysis can be in moderating the growth of long chains.

6. Coarse-Grained Models of RNA Formation Including Hydrolysis

The most straightforward way to incorporate hydrolysis into the contracted RNA models is to modify the "almost maximally contracted" model from section 4.4, by adding extra terms to the right-hand side of eqs 4.17 and 4.18. By the term "almost maximally contracted" we mean a coarse-grained reduction which describes monomers and two types of chain: short and long. (Recall that we imply here *sets* of ribonucleotide se-
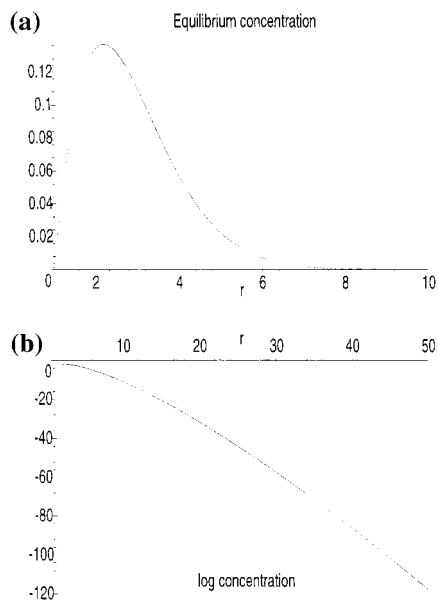
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(a)

(b)

Figure 2. Graphs of (a) concentration and (b) log (concentration) against ribonucleotide chain length (r) for the case with constant rate coefficients and constant total nucleotide density in the limit $b \to 0$, i.e., for vanishingly small hydrolysis (so that $\bar{c} = 0$).

quences, not individual RNA polymer molecules.) Then we can add in one extra mechanism—namely hydrolysis—which replaces one long chain by two short ones. However, as noted previously, since hydrolysis violates the Becker–Döring assumptions that proscribe cluster–cluster interactions, we cannot a priori build hydrolysis into a full Becker–Döring model, and then perform the coarse-graining contraction. Instead we take an already contracted model and add hydrolysis a posteriori. We return to the almost maximally contracted model described by the rate processes listed in Table 2, and denote short chains (of length Λ) by Y_n, where the subscript n enumerates the variety of different possible compositions of chains (ribonucleotide sequences), and long chains (length 2Λ) by $Y_{m,n}$. Here as before m represents the information carried in the first half of the chain and n the information in the second half. Thus the chain denoted $Y_{m,n}$ is simply a concatenation of the chain Y_m with Y_n.

We shall not analyze the full system of rate processes described in Table 2 because, using physicochemical insight, one can see immediately that some of the steps quoted there make a negligible contribution to the replication of RNA. The mechanisms which we aim to model are described in Table 3. The constant quoted after each reaction mechanism is the rate coefficient associated with that process. A number of possible mechanisms have been eliminated from our model; these are listed in Table 4.

We assume that chains are subject to hydrolysis (which splits one long chain into two short ones), and that both types of chain (short and long) are grown by an uncatalyzed mechanism as well as autocatalytically. We shall assume that chains can only act as crosscatalysts to chains of the same length or shorter; thus we neglect the putative mechanism by which long chains are formed with a short chain acting as a catalyst as the rate constant for this interaction is expected to be very small. This assumption is carried through to the ribozymic synthesis mechanisms where we assume that only longer chains can act as enzymatic catalysts.

Another assumption made in the model is that the main effect of hydrolysis is to split long chains into short ones, and not short chains into monomers. The reaction mechanism $Y_n \to \Lambda X_1$ could be added to the scheme but would further complicate the equations.

The kinetic equations for the reactions listed in Table 3 are similar to eqs 4.17 and 4.18; although slightly simpler due to the elimination of some mechanisms, they have one extra term introduced by the inclusion of hydrolysis

$$
\dot{y}_n = \sum_{m=1}^{N} \eta(y_{m,n} + y_{n,m}) -
$$
$$
\sum_{m=1}^{N} (x_1^\Lambda y_n - \beta_3 y_{m,n}) \, (\epsilon_L + \alpha_L y_{m,n} +
$$
$$
\sum_{p,q=1}^{N,N} \chi_L y_{p,q} + \sum_{p,q=1}^{N,N} \zeta_{LL} y_{m,n} y_{p,q})^\Lambda -
$$
$$
\sum_{m=1}^{N} (x_1^\Lambda y_n - \beta_3 y_{n,m}) \, (\epsilon_L + \alpha_L y_{n,m} + \sum_{p,q=1}^{N,N} \chi_L y_{p,q} +
$$
$$
\sum_{p,q=1}^{N,N} \zeta_{LL} y_{n,m} y_{p,q})^\Lambda + (x_1^\Lambda - \beta_2 y_n) \, (\epsilon_S + \alpha_S y_n + \sum_{m=1}^{N} \chi_S y_m +
$$
$$
\sum_{p,q=1}^{N,N} \chi_X y_{p,q} + \sum_{p,q=1}^{N,N} \zeta_{SS} y_{p,q} y_n + \sum_{m,p,q=1}^{N,N,N} \zeta_{SL} y_{p,q} (y_{m,n} + y_{n,m}))^\lambda
$$
$$
(6.1)
$$

$$
\dot{y}_{m,n} = -\eta y_{m,n} + (x_1^\Lambda y_m + x_1^\Lambda y_n - 2\beta_3 y_{m,n}) \, (\epsilon_L + \alpha_L y_{m,n} +
$$
$$
\sum_{p,q=1}^{N,N} \chi_L y_{p,q} + \sum_{p,q=1}^{N,N} \zeta_{LL} y_{m,n} y_{p,q})^\Lambda \quad (6.2)
$$

As a first approximation all the reactions are taken to be irreversible. Another way of interpreting this is to say that the dominant chain-shortening mechanism is hydrolysis; to leading order we ignore the reversibility of the stepwise growth mechanisms (this corresponds to setting $\beta_2 = 0 = \beta_3$).

TABLE 3. Reactions Included in the Almost Maximally Contracted Model (Equations 6.1 and 6.2) Together with Their Corresponding forward Rate Constants. Subscripts "S", "L" Denote Short and Long Chains, Respectively

$\Lambda X_1 \to Y_n$	ϵ_S	uncatalyzed polymerization
$Y_n + \Lambda X_1 \to Y_{n,m}$	ϵ_L	uncatalyzed growth of long from short chains
$Y_n + \Lambda X_1 \to 2Y_n$	α_S	autocatalysis of short chains
$Y_{m,n} + Y_m + \Lambda X_1 \to 2Y_{m,n}$	α_L	autocatalysis of long chains
$Y_{m,n} \to Y_m + Y_n$	η	hydrolysis
$Y_m + \Lambda X_1 \to Y_m + Y_n$	χ_S	crosscatalysis of short by short chains
$Y_{m,n} + Y_k + \Lambda X_1 \to Y_{m,n} + Y_{k,l}$	χ_L	crosscatalysis of long by long chains
$Y_{m,n} + \Lambda X_1 \to Y_{m,n} + Y_k$	χ_X	crosscatalysis of short by long chains
$Y_{p,q} + Y_n + \Lambda X_1 \to 2Y_n + Y_{p,q}$	ζ_{SS}	ribozymic synthesis of short chains
$Y_{p,q} + Y_{m,n} + \Lambda X_1 \to Y_n + Y_{m,n} + Y_{p,q}$	ζ_{SL}	ribozymic synthesis of short chains
$Y_{p,q} + Y_{n,m} + \Lambda X_1 \to Y_n + Y_{n,m} + Y_{p,q}$	ζ_{SL}	ribozymic synthesis of short chains
$Y_{p,q} + Y_{m,n} + Y_m + \Lambda X_1 \to 2Y_{m,n} + Y_{p,q}$	ζ_{LL}	ribozymic synthesis of long chains
$Y_{p,q} + Y_{m,n} + Y_n + \Lambda X_1 \to 2Y_{m,n} + Y_{p,q}$	ζ_{LL}	ribozymic synthesis of long chains

TABLE 4. Reactions not Included in the Model Equations 6.1 and 6.2

$Y_m + Y_k + \Lambda X_1 \rightarrow Y_m + Y_{k,l}$	χ	crosscatalysis of long by short chains
$Y_p + Y_n + \Lambda X_1 \rightarrow 2Y_n + Y_p$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{m,n} + \Lambda X_1 \rightarrow Y_n + Y_{m,n} + Y_p$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{n,m} + \Lambda X_1 \rightarrow Y_n + Y_{n,m} + Y_p$	ζ	ribozymic synthesis of short chains
$Y_p + Y_{m,n} + Y_m + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_p$	ζ	ribozymic synthesis of long chains
$Y_p + Y_{m,n} + Y_n + \Lambda X_1 \rightarrow 2Y_{m,n} + Y_p$	ζ	ribozymic synthesis of long chains

Initially we will analyze the model with constant ribonucleotide monomer concentrations, mainly because this further simplifies the system, but later we examine the constant density system. The large number and complicated nature of the nonlinear terms present in this system of equations mean that a general solution cannot be found from analytic techniques. A full solution is only available using numerical methods; however, even this is fraught with difficulty due to the combinatorially large number of equations in the system. Here we shall study special solutions analytically in order to determine certain important generic features of the kinetics allowed by these equations. As in the analysis presented in section 4.3, we shall look for uniform solutions in which all concentrations of short chains are equal, and all concentrations of long chains are equal. Once certain properties of such a solution have been established, we prove that these uniform solutions are unstable to perturbations which cause some types of chain to grow at the expense of others.

6.1. Constant Ribonucleotide Monomer Concentration. If the monomer concentration is taken as constant (pool chemical approximation) then the system of rate equations is slightly simplified. For simplicity we shall scale the concentrations so that $x_1 = 1$. As noted above, these assumptions imply that $\beta_2 = 0 = \beta_3$.

To find the uniform solution, for which the concentrations of all short chains are equal, and similarly for the long chains, we assume that all the short chains $y_n(t)$ have the same concentration (say $Y(t)$) and all the long chains another concentration, $Z(t) = y_{m,n}(t)$. With these assumptions the very large system of rate equations (eqs 6.1 and 6.2) reduces to a coupled pair of ordinary differential equations

$$\dot{Y} = 2\eta NZ + (\epsilon_S + \alpha_S Y + \chi_S NY + \chi_X N^2 Z + \zeta_{SS} N^2 YZ + 2\zeta_{SL} N^3 Z^2)^\lambda$$

$$- 2NY(\epsilon_L + \alpha_L Z + \chi_L N^2 Z + \zeta_{LL} N^2 Z^2)^\Lambda \quad (6.3)$$

$$\dot{Z} = -\eta Z + 2Y(\epsilon_L + \alpha_L Z + \chi_L N^2 Z + \zeta_{LL} N^2 Z^2)^\Lambda \quad (6.4)$$

It is not possible to find a steady-state solution of these equations, since setting $\dot{Z} = 0$ in eq 6.4 implies that the flux from long to short chains caused by hydrolysis exactly balances the growth of long from short. Such a balance in eq 6.3 in turn implies that $\dot{Y}$ must be strictly positive, removing any possibility of a physically relevant equilibrium configuration existing. Indeed, as this system evolves, the concentration of long chains grows without bound, and the concentration of short chains decreases to zero. The catalytic properties of chain growth dominate the system, leaving hydrolysis as a negligible effect, to the extent that there is a finite time singularity. The leading order balance in eq 6.3 is $2^\lambda \zeta_{SL}^\lambda N^{3\lambda} Z^{2\lambda} \sim 2Y \zeta_{LL}^\Lambda N^{2\Lambda} Z^{2\Lambda}$, implying that, as $Z \rightarrow \infty$, $Y \sim 1/\sqrt{Z}$. Then eq 6.4 implies $\dot{Z} \sim Z^{2\lambda}$, and so $Z \sim (1 - t/t_c)^{1/(2\lambda-1)}$. The strong catalytic properties of long chains coupled with irreversible polymerization reactions cause the rapid growth in the concentration of long chains to

occur. If we were to introduce reversible reactions ($\beta_2 \neq 0 \neq \beta_3$), then the singularity would be removed. This modification would also cause a uniform steady-state to appear, in which all the concentrations of short chains were equal, and all the concentrations of long chains were equal.

A singularity of the form described above is also present in the full system of eqs 6.1 and 6.2 with $\beta_2 = 0 = \beta_3$. This case is thus rather unphysical, so we shall turn to the situation where density is conserved: an assumption which is more physically relevant and will also eliminate the singularity encountered above.

6.2. Constant Total Concentration of Ribonucleotides in the Soup. The full kinetic equations for the case where all the catalytic growth reactions are irreversible ($\beta_2 = \beta_3 = 0$) and density (ρ) is kept constant are

$$\dot{y}_{m,n} = -\eta y_{m,n} + (y_m + y_n)\left(\rho - \Lambda\sum_{p=1}^{N} y_p - \right.$$
$$\left. 2\Lambda\sum_{p,q=1}^{N,N} y_{p,q}\right)^\Lambda \left(\epsilon_L + \alpha_L y_{m,n} + \sum_{p,q=1}^{N,N}\chi_L y_{p,q} + \sum_{p,q=1}^{N,N}\zeta_{LL}y_{p,q}y_{m,n}\right)^\Lambda$$
$$(6.5)$$

$$\dot{y}_n = \sum_{m=1}^{N}\eta(y_{m,n} + y_{n,m}) - \sum_{m=1}^{N}y_n(\rho - \Lambda\sum_{p=1}^{N}y_p -$$
$$2\Lambda\sum_{p,q=1}^{N,N}y_{p,q})^\Lambda(\epsilon_L + \alpha_L y_{m,n} + \sum_{p,q=1}^{N,N}\chi_L y_{p,q} +$$
$$\sum_{p,q=1}^{N,N}\zeta_{LL}y_{p,q}y_{m,n})^\Lambda - \sum_{m=1}^{N}y_n(\rho - \Lambda\sum_{p=1}^{N}y_p -$$
$$2\Lambda\sum_{p,q=1}^{N,N}y_{p,q})^\Lambda(\epsilon_L + \alpha_L y_{n,m} + \sum_{p,q=1}^{N,N}\chi_L y_{p,q} +$$
$$\sum_{p,q=1}^{N,N}\zeta_{LL}y_{p,q}y_{n,m})^\Lambda + (\rho - \Lambda\sum_{p=1}^{N}y_p -$$
$$2\Lambda\sum_{p,q=1}^{N,N}y_{p,q})^\Lambda(\epsilon_S + \alpha_S y_n + \sum_{m=1}^{N}\chi_S y_m + \sum_{p,q=1}^{N,N}\chi_X y_{p,q} +$$
$$\sum_{p,q=1}^{N,N}\zeta_{SS}y_{p,q}y_n + \sum_{m,p,q=1}^{N,N,N}\zeta_{SL}y_{p,q}(y_{m,n} + y_{n,m}))^\lambda \quad (6.6)$$

where it will be recalled that $N = 4^\Lambda$, the number of sequences of length Λ. Of course, even this set of coupled equations cannot be solved exactly either analytically (due to its nonlinearity) or numerically (since it is NP hard). However, we can make important progress by using theoretical methods to study certain special solutions and their local behavior.

6.2.1. Approach to the Equilibrium Solution. The simplest solutions of all are the time-independent ones, and in this case there is a family of equilibrium solutions. These equilibrium solutions are degenerate, in that only short chains are present; there are no monomers or long chains. It is thus a genuine equilibrium state since there are no fluxes present in the system.

The reason for the time-independent solution being a true equilibrium solution rather than a non-equilibrium steady-state is that once all monomers have been converted into chains (whether short or long), no further growth can occur; the only remaining process is hydrolysis, which converts all the long chains into shorter oligomers. Hence as $t \rightarrow \infty$, the system approaches

$$\bar{y}_{m,n} = 0, \quad \bar{x}_1 = 0, \quad \bar{y}_m \geq 0 \qquad \text{such that}$$

$$\sum_{m=1}^{N} \bar{y}_m = \rho/\Lambda \quad (6.7)$$

which is an arbitrary distribution of short chains satisfying mass conservation. Thus the kinetics allowed for in the model permit highly non-uniform equilibrium solutions, including the extinction of species (that is all the longer chains, and any number of the shorter chains).

Expanding the dynamical behavior about the equilibrium solution, we can find the late-time asymptotics which tell us how such a self-reproducing soup of ribonucleotides approaches an equilibrium state. The quantities $y_{m,n}(t)$, $x_1(t)$ both tend to zero, and we put $y_n(t) = \bar{y}_n - h_n(t)$ with $h_n(t) \rightarrow 0$ as $t \rightarrow \infty$. To leading order, the quantities h_n, x_1, and $y_{m,n}$ then satisfy

$$x_1 = \Lambda \left(\sum_{q=1}^{N} h_q - 2 \sum_{p,q=1}^{N,N} y_{p,q} \right) \quad (6.8)$$

$$\dot{y}_{m,n} = -\eta y_{m,n} + \epsilon_L^{\Lambda} \Lambda^{\Lambda} (\bar{y}_m + \bar{y}_n) \left(\sum_{q=1}^{N} h_q - \sum_{p,q=1}^{N,N} y_{p,q} \right)^{\Lambda} \quad (6.9)$$

$$\dot{h}_n = -\eta \sum_{m=1}^{N} (y_{m,n} + y_{n,m}) - \Lambda^{\Lambda} \left(\sum_{q=1}^{N} h_q - 2 \sum_{p,q=1}^{N,N} y_{p,q} \right)^{\Lambda} \left[\left(\epsilon_S + \alpha_S \bar{y}_n + \frac{\chi_S \rho}{\Lambda} \right)^{\lambda} - 2N\epsilon_L^{\Lambda} \bar{y}_n \right] \quad (6.10)$$

As in our earlier study of self-reproducing vesicles,[22] the phase space trajectories followed by the mathematical solutions of these kinetic equations are not strongly attracted to an equilibrium configuration; rather the dynamics close to equilibrium occurs on a *center manifold*, where nonlinear terms dominate the behavior. Motion on this manifold occurs slowly: relaxation does not follow an exponential decay with a characteristic time scale (relaxation time); rather the concentrations vary as inverse powers of time, with exponents $-1/\lambda$ for short chains and monomers and $-\Lambda/\lambda$ for long chains.

To explicitly find the nature of the approach to equilibrium, we substitute

$$x_1(t) = \tilde{x}_1 t^{-1/\lambda}, \quad h_n(t) = \tilde{h}_n t^{-1/\lambda}, \quad y_{m,n}(t) = \tilde{y}_{m,n} t^{-\Lambda/\lambda} \quad (6.11)$$

into eqs 6.8−6.10. This yields

$$\tilde{x}_1 = \Lambda \tilde{H}, \quad \tilde{y}_{m,n} = \frac{\epsilon_L^{\Lambda} \Lambda^{\Lambda} \tilde{H}^{\Lambda} (\bar{y}_m + \bar{y}_n)}{\eta},$$

$$\tilde{h}_n = \lambda \Lambda^{\Lambda} \tilde{H}^{\Lambda} \left[\frac{2\rho \epsilon_L^{\Lambda}}{\Lambda} + \left(\epsilon_S + \alpha_S \bar{y}_n + \frac{\rho \chi_S}{\Lambda} \right)^{\lambda} \right] \quad (6.12)$$

where $\tilde{H} = \sum_{q=1}^{N} \tilde{h}_q$. The latter expression enables $\tilde{H}$ to be found:

$$\frac{1}{\tilde{H}^{\lambda}} = \lambda \Lambda^{\Lambda} \left[\frac{2\epsilon_L^{\Lambda} \rho N}{\Lambda} + \sum_{q=1}^{N} \left(\epsilon_S + \alpha_S \bar{y}_q + \frac{\rho \chi_S}{\Lambda} \right)^{\lambda} \right] \quad (6.13)$$

These results show that the short chains with largest equilibrium concentration $\bar{y}_n$ also have the fastest growth rates near equilibrium, since the larger $\bar{y}_n$, the larger $\tilde{h}_n$. Note, however, that this is only a *local* analysis: we have only found how the system behaves once it is close to any one of its degenerate equilibrium solutions; we have not yet discovered anything about which equilibrium solution is approached or how it evolves from an initial condition towards equilibrium. To gain more insight about earlier times in the evolution of the system, we now analyze a special solution−the uniform solution.

6.2.2. Uniform Solution and Its Stability. We now turn to analyze more general, far from equilibrium, kinetics; in particular, we shall be concerned with investigating whether the uniform growth of all chains is a stable configuration. The *Ansatz*

$$y_{m,n} = Z(t), \quad y_m(t) = Y(t) \quad (6.14)$$

when inserted into eqs 6.5 and 6.6, implies

$$\dot{Z} = -\eta Z + 2Y (\rho - \Lambda NY - 2\Lambda N^2 Z)^{\Lambda} (\epsilon_L + \alpha_L Z + \chi_L N^2 Z + \zeta_{LL} N^2 Z^2)^{\Lambda} \quad (6.15)$$

$$\dot{Y} = 2\eta NZ - 2NY (\rho - \Lambda NY - 2\Lambda N^2 Z)^{\Lambda} (\epsilon_L + \alpha_L Z + \chi_L N^2 Z + \zeta_{LL} N^2 Z^2)^{\Lambda} + (\rho - \Lambda NY - 2\Lambda N^2 Z)^{\Lambda} (\epsilon_S + \alpha_S Y + \chi_S NY + \chi_X N^2 Z + \zeta_{SS} N^2 YZ + 2\zeta_{SL} N^3 Z^2)^{\lambda} \quad (6.16)$$

These equations are not explicitly solvable in terms of elementary functions due to the number and strength of the nonlinear terms present. The *Ansatz* has, however, removed the NP hard complexity of eqs 6.5 and 6.6 so, if desired, a numerical solution of eqs 6.15 and 6.16 could be found using standard methods. We shall not pursue this here, as it is our aim to show that the solution is in fact unstable and so will not be realized. The presence of an instability means that any small perturbation of the solution will grow as time progresses, and the full system eqs 6.5 and 6.6 will not approach the equilibrium solution of eqs 6.15 and 6.16. In our system of equations we shall assume that the instability is initiated by slightly nonuniform initial conditions; in a more general model the instability could alternatively be caused by different chain types having slightly different reaction rates. We shall also demonstrate that the instability is effective at earlier times for certain choices of parameters, indicating that the uniform solution will not be manifested in the solution of eqs 6.5 and 6.6.

To illustrate this instability mathematically, we perform a linear expansion about the uniform solution, putting $y_{m,n} = Z + Z\hat{y}_{m,n}$ and $y_n = Y + Y\hat{y}_n$, with $\hat{y}_n, \hat{y}_{m,n} \ll 1$, into eqs 6.5 and 6.6. At leading order, this produces

$$\dot{\hat{y}}_n = A(t)\hat{y}_n + B(t) \sum_{m=1}^{N} (\hat{y}_{m,n} + \hat{y}_{n,m}) \quad (6.17)$$

$$\dot{\hat{y}}_{m,n} + C(t)\hat{y}_{m,n} + D(t)[\hat{y}_m + \hat{y}_n] \quad (6.18)$$

where

$$A(t) = -\frac{2N\eta Z}{Y} + \frac{1}{Y}[(\lambda - 1)(\alpha_S + N^2\zeta_{SS}Z)Y - $$
$$(\epsilon_S + N\chi_S Y + N^2\chi_X Z + 2\zeta_{SL}N^3Z^2)] (\rho - \Lambda NY - $$
$$2\Lambda N^2 Z)^\Lambda (\epsilon_S + \alpha_S Y + \chi_S NY + \chi_X N^2 Z + \zeta_{SS}N^2 YZ + $$
$$2\zeta_{SL}N^3Z^2)^{\lambda-1}$$

$$B(t) = \frac{\eta Z}{Y} - \Lambda Z(\alpha_L + \zeta_{LL}N^2Z)(\rho - \Lambda NY - 2\Lambda N^2Z)^\Lambda (\epsilon_L + $$
$$\alpha_L Z + \chi_L N^2 Z + \zeta_{LL}N^2Z^2)^\lambda + \frac{\lambda\zeta_{SL}N^2Z^2}{Y}(\rho - \Lambda NY - $$
$$2\Lambda N^2 Z)^\Lambda (\epsilon_S + \alpha_S Y + \chi_S NY + \chi_X N^2 Z + \zeta_{SS}N^2 YZ + $$
$$2\zeta_{SL}N^3Z^2)^{\lambda-1}$$

$$C(t) = \frac{2Y}{Z}(\rho - \Lambda NY - 2\Lambda N^2Z)^\Lambda (\epsilon_L + \alpha_L Z + \chi_L N^2 Z + $$
$$\zeta_{LL}N^2Z^2)^\lambda [\lambda Z(\alpha_L + \zeta_{LL}N^2Z) - (\epsilon_L + \chi_L N^2Z)]$$

$$D(t) = \frac{Y}{Z}(\rho - \Lambda NY - 2\Lambda N^2Z)^\Lambda (\epsilon_L + \alpha_L Z + \chi_L N^2 Z + $$
$$\zeta_{LL}N^2Z^2)^\Lambda \quad (6.19)$$

Now it can be shown that if $\sum_{m=1}^{N} \hat{y}_m = 0 = \sum_{p,q=1}^{N,N} \hat{y}_{p,q}$ at any instant in time, then these quantities are zero for all time. Since it is sufficient to consider perturbations of such a form, this result has been used to simplify the above expressions.

Since eqs 6.17 and 6.18 are linear in $\hat{y}_n$, $\hat{y}_{m,n}$, the temporal dimension of the problem can be separated from the compositional part by expressing the solution in the form

$$\hat{y}_n(t) = \xi_n S(t), \quad \hat{y}_{m,n}L(t) = \xi_{m,n}L(t) \quad (6.20)$$

This approach introduces a new parameter into the problem—the separation constant K, where $\xi_{m,n} = K(\xi_m + \xi_n)$. Proving that the uniform solution is unstable then reduces to demonstrating that the functions $L(t)$ and $S(t)$ grow as time (t) increases. These functions satisfy the differential equations

$$\begin{pmatrix} \dot{S} \\ \dot{L} \end{pmatrix} = \begin{pmatrix} A(t) & 2N K B(t) \\ D(t)/K & C(t) \end{pmatrix} \begin{pmatrix} S \\ L \end{pmatrix} \quad (6.21)$$

From this it is possible to deduce that the uniform solution is unstable for certain choices of the parameters. A detailed analysis is hindered by the fact that the uniform solution for $Y(t)$ and $Z(t)$ (eqs 6.15 and 6.16) cannot be explicitly determined.

The simplest part of the reaction kinetics to analyze is the approach to equilibrium, and this is where the most rigorous results can be obtained. We shall discuss this stage of the reaction first, and then proceed to derive more general and approximate results for earlier stages of the polymerization scheme.

6.2.3. Instability in the Approach to Equilibrium. The only equilibrium solution of the system (eqs 6.15 and 6.16) is $\bar{Z} = 0$ and $\bar{Y} = \rho/N\Lambda$ which implies $\bar{x}_1 = 0$. The approach to this state is governed by

$$x_1(t) \sim \Lambda NY_1 t^{-1/\lambda}, \quad Y(t) \sim \frac{\rho}{\Lambda N} - Y_1 t^{-1/\lambda}, \quad Z(t) \sim Z_1 t^{-\Lambda/\lambda},$$
$$\text{as } t \to \infty \quad (6.22)$$

where $Y_1, Z_1 > 0$ can be found either from an expansion of eqs 6.15 and 6.16 about the equilibrium solution, or by inserting the *Ansatz* (eq 6.14) into eqs 6.12 and 6.13; they are thus determined by

$$\frac{1}{Y_1^\lambda} = \lambda\Lambda^\lambda N^\lambda \left\{ 2\rho\epsilon_L^\Lambda + \Lambda\left(\epsilon_S + \frac{\rho\alpha_S}{\Lambda N} + \frac{\rho\chi_S}{\Lambda}\right)^\Lambda \right\},$$

$$Z_1 = \frac{2\rho\Lambda^\lambda N^\lambda \epsilon_L^\Lambda Y_1^\Lambda}{\eta} \quad (6.23)$$

An examination of eqs 6.17 and 6.19 in the limit $t \to \infty$ highlights one condition for an instability to exist. In this limit, the kinetic equations simplify to

$$t^{\Lambda/\lambda}\dot{\hat{y}}_n = A_0\hat{y}_n + 2(\Lambda NY_1\epsilon_L)^\Lambda \sum_{m=1}^{N} (\hat{y}_{m,n} + \hat{y}_{n,m}),$$

$$\dot{\hat{y}}_{m,n} = \frac{1}{2}\eta(\hat{y}_m + \hat{y}_n - \hat{y}_{m,n}) \quad (6.24)$$

where

$$A_0 = (N\Lambda Y_1)^\Lambda\left\{\left[(\lambda - 1)\alpha_S - N\left(\frac{\Lambda\epsilon_S}{\rho} + \chi_S\right)\right]\left(\epsilon_S + \frac{\alpha_S\rho}{N\Lambda} + \frac{\chi_S\rho}{\Lambda}\right)^{\lambda-1} - 4N\epsilon_L^\Lambda\right\} \quad (6.25)$$

Separating the temporal and compositional variables, as in eq 6.20, we obtain a simplified system of equations which can be written as

$$t^{\Lambda/\lambda}\frac{d^2L}{dt^2} + (\eta t^{\Lambda/\lambda} - A_0)\frac{dL}{dt} - \eta[A_0 + 2N(\epsilon_L\Lambda NY_1)^\Lambda]L = 0 \quad (6.26)$$

Unfortunately, it is not possible to solve this equation analytically, but the closely related system

$$t\frac{d^2L}{dt^2} + (\eta t - A_0)\frac{dL}{dt} - \eta[A_0 + 2N(\epsilon_L\Lambda NY_1)^\Lambda]L = 0 \quad (6.27)$$

can be solved in terms of hypergeometric functions (see Abramowitz and Stegun[28] for details). This approximation is easily justified by noting that the range of chain lengths we are interested in is $\Lambda = \lambda + 1$ to be approximately 50. The solution of eq 6.27 can be written as

$$L(t) = K_1 M(-2N(\epsilon_L\Lambda NY_1)^\Lambda - A_0, -A_0, -\eta t) + K_2 U(-2N(\epsilon_L\Lambda NY_1)^\Lambda - A_0, -A_0, -\eta t) \quad (6.28)$$

where K_1 and K_2 are constants of integration and the functions $M(\cdot)$ and $U(\cdot)$ are as defined in Chapter 13 of Abramowitz and Stegun.[28] This solution grows in the limit $t \to \infty$ if $A_0 + 2N(\epsilon_L\Lambda NY_1)^\Lambda > 0$. This condition can be rewritten as

$$\left[(\lambda - 1)\alpha_S - N\left(\frac{\epsilon_S\Lambda}{\rho} + \chi_S\right)\right]\left(\epsilon_S + \frac{\alpha_S\rho}{N\Lambda} + \frac{\chi_S\rho}{\Lambda}\right)^\Lambda > 2N\epsilon_L^\Lambda \quad (6.29)$$

Thus the instability depends on the autocatalytic rate being greater than the sum of the uncatalyzed rate and crosscatalyzed (copying error) rate by a factor which depends on the number of chains.

Qualitatively, this agrees with our intuition: in order for perturbations to grow, *the autocatalytic rate should be greater than the total rate at which error-prone copies are produced* (whether that be by the uncatalyzed or the catalyzed mechanism).

6.2.4. More General Instability Analysis of the Uniform Solution. Formally, the Routh−Hourwitz criteria[29] for eq 6.21 are that the system is stable if $A + C < 0$, and $AC > 2NBD$. Therefore, to prove that the system is unstable our aim is to prove at least one of

$$A + C > 0, \quad AC < 2NBD \qquad (6.30)$$

holds. However, since A, B, C, and D are functions of time, results based on these inequalities rely on interpreting stability in the looser manner of "as t increases the perturbation grows", rather than a strict argument in the style of "as $t \to \infty$, $S(t)$, and $L(t) \sim f(t)$, for some given $f(t)$". More rigorous results in the limit $t \to \infty$ have already been given in section 6.2.3.

One interesting point from a mathematical perspective is that the stability criteria do not depend on the separation parameter K. Thus the bifurcation where the transition from stable to unstable behavior occurs is degenerate in that many modes of the system change stability at the same point in parameter space.

The Routh−Hourwitz analysis confirms the presence of an instability as the system (eq 6.21) approaches equilibrium, but since we are now working with a slightly different definition of stability and have not made the approximation by which eq 6.26 is replaced by eq 6.27, we obtain a slightly different inequality

$$\alpha_S > \frac{N(\Lambda \epsilon_S + \rho \chi_S)}{(\lambda - 1)\rho} \qquad (6.31)$$

This inequality can be interpreted in a number of different ways; for example, it sets upper limits on the uncatalyzed growth rates for chains ϵ_S. Another interpretation is that it prescribes an upper limit on the error-prone self-replication rates χ_S. The limits, however depend on the chain length (N), so a third explanation is that it sets a maximum on N, the length of chain which can successfully self-replicate and maintain its concentration at a significantly higher level than other chains of the same length. Finally, it prescribes a minimum for the autocatalytic rate constant above which the differences in concentration between chains grow.

We progress now to apply the inequalities (eq 6.30) to special cases of the system (eqs 6.17−6.19), since it is then simpler to draw more specific conclusions. The special cases we consider are (i) systems with only crosscatalysis and autocatalysis and (ii) systems where ribozymic synthesis dominates chain polymerization. Of course in the RNA world both processes occur simultaneously and interact with each other; only an analysis of the full system (eqs 6.17−6.19) will reveal how these mechanisms affect each other, but some useful preliminary insight and results are gained from analyzing the processes independently.

6.2.5. Effect of Template-Based Catalysis on the Uniform Solution. To analyze the effect of standard template-based synthesis on the process, we set all the other rate constants to zero, $\epsilon_L = \epsilon_S = \zeta_{SS} = \zeta_{SL} = \zeta_{LL} = 0$ leaving only α_L, α_S, χ_S, χ_X, χ_L, and $\eta \neq 0$. We then solve the model subject to initial conditions in which only a few chains are present ($0 < Y(0)$ and $Z(0) \ll 1$). The condition $A + C > 0$ can then be simplified to

$$(\alpha_S Y + N\chi_S Y + N^2\chi_X Z)^{\lambda-1}[(\lambda - 1)\alpha_S Y - N\chi_S Y - N^2\chi_X Z] + 2Y^2 Z^\lambda(\alpha_L + N^2\chi_L)^\lambda[\lambda\alpha_L - N^2\chi_L] >$$
$$\frac{2N\eta Z}{(\rho - \Lambda NY - 2\Lambda N^2 Z)^\Lambda} \qquad (6.32)$$

This inequality is highly suggestive of the simple conditions

$$(\lambda - 1)\alpha_S > N\chi_S, \quad \lambda\alpha_L > N\chi_L \qquad (6.33)$$

Since the right-hand side of eq 6.32 is positive, one or both of the terms on the left-hand side must be too. Requiring the first term to be positive implies the first part of eq 6.33 and the second term in eq 6.32 yields the second. If both parts of eq 6.33 hold then for small enough η an instability will exist. Such inequalities are fully in accord with earlier results (eqs 6.29 and 6.31) as well as our intuition that autocatalysis should dominate crosscatalysis in order for distinct species of chains to emerge and remain viable.

The other condition ($AC < 2NBD$) can be rewritten as

$$[\lambda\alpha_L - N^2\chi_L][(\lambda - 1)\alpha_S Y - N\chi_S Y - N^2\chi_X Z](\alpha_S Y + N\chi_S Y + N^2\chi_X Z)^{\lambda-1} + N\Lambda\alpha_L YZ^\Lambda(\alpha_L + N^2\chi_L)^\Lambda <$$
$$\frac{N\eta Z[(\Lambda + \lambda)\alpha_L - N^2\chi_L]}{(\rho - \Lambda NY - 2\Lambda N^2 Z)^\Lambda} \qquad (6.34)$$

which shows that the presence of hydrolysis (the η term) can cause an instability. Note that while the previous inequality gave an upper limit for η, this inequality prescribes a lower bound. At this stage, it is perhaps worth repeating that only one of the two inequalities (eqs 6.32 and 6.34) need be satisfied for an instability in the system to exist.

Simple inequalities such as eqs 6.31 and 6.33 are actually quite stringent; since N is exponentially large in Λ, the autocatalytic reaction rate must far exceed that of the cross-catalytic rate. It is helpful to define a quality factor Q for these template-based chain synthesis mechanisms. There are N crosscatalytic processes in our model, each operating at the rate χ, only one of which produces an accurate copy of the catalyst, and there is one autocatalytic mechanism which is taken to provide an exact replication of the catalyst, at rate α. Thus from all the template-based catalytic chain synthesis processes the proportion of accurate copies is

$$Q = \frac{\alpha + \chi}{\alpha + N\chi} \qquad (6.35)$$

This quantity can be defined for the formation of short or long chains. The inequalities given in 6.31 and 6.33 can be recast into conditions on Q, in the manner of Nuño *et al.*,[16] and in this form, they do not appear so demanding. For example, the second of the inequalities in eq 6.33 becomes

$$Q > \frac{1}{\Lambda} + \frac{\lambda}{\Lambda N} \qquad (6.36)$$

By considering these template-based catalytic processes along with uncatalyzed RNA polymerization (that is, ignoring ribozymic synthesis) it is possible to make a crude estimate of the timescales over which RNA chain formation occurs. Integrating a simplified but dimensional version of eq 6.3

$$\dot{Y} = \rho^\Lambda(\epsilon_S + \alpha_S Y)^\lambda \qquad (6.37)$$

we find

$$t \approx \frac{1}{(\lambda - 1)\rho^\Lambda \epsilon_S^{\lambda-1} \alpha_S} \qquad (6.38)$$

Taking a chain length of $\Lambda = 10$, a concentration (density) of $\rho = 10^{-3}$ M and $\alpha = 10^5 \epsilon_S$, we obtain for the possible number

of chain sequences $N = 10^6$. The time scale given in eq 6.38 then gives a quantity which is consistent with RNA chains forming in months to years, if the value chosen for ϵ_S is taken to lie in the range $3.5-4.5$ M^{-1} s$^{-1/\lambda}$. A much faster time scale is obtained if one calculates the rate of divergence from the uniform solution according to $A(t)$ in eq 6.17. *Thus the model shows that the selection of one species over another happens on a much faster time scale than the growth of chains.* These estimates are crude due to their reliance on raising the reaction rate ϵ_S and density ρ to the power Λ; for example, with $\Lambda = 10$, an alteration of a parameter by a factor of two yields a factor of $2^{10} \approx 10^3$ in the final approximation for the time scale.

6.2.6. Effect of Ribozymic Synthesis on the Uniform Solution. It is harder to derive concise results from the case of ribozymic synthesis since these mechanisms introduce higher nonlinearities and coupling between different chains than the usual catalytic mechanisms.

In this case we set all catalytic and normal rate constants to zero ($\epsilon_L = \epsilon_S = \alpha_L = \alpha_S = \chi_S = \chi_X = \chi_L = 0$) and keep only the hydrolysis and ribozymic synthesis rates nonzero (η, ζ_{SS}, ζ_{SL}, ζ_{LL}), cf. Table 3. The condition $A + C > 0$ (eq 6.30) then reduces to

$$2\lambda\zeta_{LL}^\Lambda N^2 Y^2 Z^{\Lambda+1} + (\zeta_{SS}Y + 2\zeta_{SL}NZ)^{\lambda-1}[(\lambda-1)\zeta_{SS}Y - 2\zeta_{SL}NZ] > \frac{2\eta N^{1-2\lambda}Z^{1-\lambda}}{(\rho - \Lambda NY - 2\Lambda N^2 Z)^\Lambda} \quad (6.39)$$

which clearly fails when Z is small and when the monomer concentration is small, since in both these cases the right-hand side becomes large. However at intermediate times this inequality can be satisfied. For example, if ζ_{SS} is sufficiently larger than ζ_{SL}, then the second term could become large enough to dominate the right-hand side. This condition shows that the highly specific nature of ribozyme-assisted synthesis of a short chain by an identical or complimentary short chain is of greater importance to the instability than the less selective synthesis of a short chain by a long chain. Alternatively, the first term indicates that a large value of ζ_{LL} (ribozyme-assisted synthesis of long chains by long chains) can instigate the instability.

The other instability condition $AC < 2NBD$ reduces to

$$\frac{\lambda}{N}(\zeta_{SS}Y + 2\zeta_{SL}NZ)^{\lambda-1}[(\lambda-1)\zeta_{SS}Y - 2\zeta_{SL}NZ] +$$

$$N^2\Lambda\zeta_{LL}^\Lambda YZ^{\Lambda+1} < \frac{\eta(\Lambda+\lambda)N^{-2\lambda}Z^{1-\lambda}}{(\rho - \Lambda NY - 2\Lambda N^2 Z)^\Lambda} + \lambda\zeta_{SL}Z(\zeta_{SS}Y + 2\zeta_{SL}NZ)^{\lambda-1} \quad (6.40)$$

In the limits $Z \rightarrow 0$ and $x_1 \rightarrow 0$ this inequality is satisfied, implying the system is unstable in these limits even though the previous inequality 6.39 is not satisfied. Due to the large size of N, we expect $N^{-2\lambda}$ to be insignificant, and so if this inequality were to hold, it would be mainly due to the presence of ribozyme-assisted synthesis of short chains by long—the ζ_{SL} term.

7. Discussion

The question of which type of RNA chain (one ribonucleotide sequence or a set of such sequences) will grow from an initial soup of monomers presents an extremely difficult challenge both experimentally and theoretically. From the latter perspective with which we have been concerned in this paper, it is a massively degenerate version of the problem of polymorphism, that is, of determining which particular crystal structure among several

will grow from a melt or supersaturated solution. This problem is known to depend on the kinetics of the process—it is not an equilibrium phenomena: the thermodynamically most stable macroscopic structure is not necessarily the one which nucleates first or grows the fastest, as is well known in the fields of biomineralization[30] and the crystallization of macromolecules.[31,32] In the present context, the difficulty is compounded many times over owing to the combinatorial complexity of polyribonucleotide self-reproduction, which causes the problem to acquire an NP hard character in the language of algorithmic complexity theory.[4]

The models we have introduced and analyzed in this paper include many catalytic mechanisms which affect the rate at which various reactions proceed; these include inter alia template-based and ribozymal replicase-assisted RNA synthesis. Our paper has addressed a basic question concerning the putative origins of the RNA world from the standpoint of plausible chemical kinetics and provides important evidence supporting the notion that self-reproducing ribonucleotide polymers could quite easily have emerged from a prebiotic soup. In reaching this conclusion, we have analyzed carefully the nonlinearities with which this problem is replete. It is worth pointing out that much of the previous work on related issues, including that of Eigen et al. on hypercycles,[1,10-12] was based on the analysis of *linearized* kinetic equations, a procedure that drastically suppresses the difficulty of the problem together with the richness of possible behavior. Nuño et al.'s work, like that of Eigen et al., also assumes the existence of self-reproducing hypercycles, but does incorporate certain nonlinearities.[15,16] Addressing a different problem, they model template-based synthesis using quadratic nonlinearities as in our models. In addition, our models, include ribozymically assisted synthesis described by cubic nonlinearities. However, in our approach the coarse-graining contraction procedure, used to overcome the combinatorial explosion of possible proliferating polyribonucleotide sequences, introduces extremely high nonlinearities into the analysis. Overall, it is the key combination of *hydrolysis and catalysis* which we find produces stable populations of a small number of selected chain types.

Cross-catalysis (error-prone template-based copying) speeds up self-reproduction generally, but it tends to even out the distribution of sequences since it acts non-specifically. Autocatalysis and hydrolysis are sequence specific. Hydrolysis splits one chain into two: whereas a single long chain can catalyze only one reaction, the two shorter chains produced by hydrolysis can each catalyse reactions which make chains similar to the original long chain. Thus hydrolysis aids the autocatalytic replication process by recycling and hence multiplying the number of chains of a given length capable of autocatalysis. This recycling of material and information can be thought of as primordial metabolism. If hydrolysis acts more slowly than autocatalysis there is not enough catalytic material around to maintain the instability driving the preferential formation of certain sequences and the suppression of others; then other factors will even out the differences in concentration of different species and no selectivity ensues.

The results of section 6 show that even with hydrolysis, the presence of catalytic chain growth mechanisms implies that viable concentrations of long chains will eventually be formed if one is prepared to wait long enough. At the end of section 6.2.5, a simple calculation was carried out to estimate the length of this induction time. Although estimates of numerical values of our parameters are extremely hard to find, the data available

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is consistent with a time scale of months for the formation of viable concentrations of chains of 10 bases.

In conclusion, we have demonstrated that it is possible to realize the selection of certain self-replicating RNA polymer chains in a reasonable amount of time starting from plausible assumptions about the chemistry and initial conditions that could have prevailed within a putative prebiotic soup comprised of β-D-ribonucleotide monomers.

Appendix A. Analysis of an Alternative Kinetic Model for Pure Autocatalytic RNA Replication without Hydrolysis

In section 3.2, we considered a detailed kinetic model for RNA replication that omitted hydrolysis and assumed that rate coefficients for polymerization were independent of chain length. In this section we retain the same structure for the kinetic eq 3.14, but instead of assuming that the reaction rate coefficients are independent of chain length as in eq 3.17, here we make them proportional to chain length: $\alpha_{r,s} = rs$, $\alpha_{r,0} = \epsilon r$. The flux eqs 3.17 are thus replaced by

$$Jr^{\gamma, N_i} = c_r^\gamma c_1^{N_i}\left(\epsilon r + \sum_\theta rsc_s^\theta\right), \quad J_r^{N_i,\gamma} = c_r^\gamma c_1^{N_i}\left(\epsilon r + \sum_\theta rsc_s^\theta\right) \tag{A1}$$

and inserted into the kinetic eqs 3.14 (hydrolysis is again neglected). We continue ignore the exact order of nucleotides in the chains γ, concentrating purely on the length of chains, in order to investigate kinetics by which a distribution of long chains may be formed. We define $f_r = \sum_{\gamma:|\gamma|=r} c_r^\gamma$ in the same sense as before to simplify the governing eqs 3.14 and A1 to

$$\dot{f}_r = 2f_1 f_{r-1}\left(\epsilon(r-1) + \sum_s (r-1)s f_s\right) - 2f_1 f_r \left(\epsilon r + \sum_s rsf_s\right) \tag{A2}$$

As in the previous example, the system of ordinary differential equations can be solved using the generating function eq 3.19, which now satisfies the partial differential equation

$$\frac{\partial F}{\partial t} - 2f_1\frac{\partial F}{\partial z}(1 - e^{-z})(\epsilon - G_0(t)) = 2f_1^2 e^{-z}(\epsilon - G_0(t)) \tag{A3}$$

where $G_0(t) = \partial F/\partial z|_{z=0}$ (thus $G_0(0) = -f_1$).

Equation A3 is significantly harder to solve than the previous example; it is best analyzed using the method of characteristics. First, however, we shall find the function $G_0(t)$. We define $G(z, t) = \partial F/\partial z$ so that if $G_0(t) = G(0, t)$, then eq A3 implies $G(z, t)$ satisfies the equation

$$\frac{\partial G}{\partial t} = 2f_1(1 - e^{-z})(\epsilon - G_0)\frac{\partial G}{\partial z} - e^{-z}(\epsilon - G_0)G = -f_1 e^{-z}(\epsilon - G_0) \tag{A4}$$

In the limit $z \to 0$ this reduces to the ODE

$$\frac{dG_0}{dt} = 2f_1[\epsilon - G_0(t)][G_0(t) - f_1] \Rightarrow$$
$$G_0(t) = f_1\left(\frac{(f_1 + \epsilon)e^{2tf_1(f_1-\epsilon)} - 2\epsilon}{(f_1 + \epsilon)e^{2tf_1(f_1-\epsilon)} - 2f_1}\right) \tag{A5}$$

when integrated using the initial condition $G_0(0) = -f_1$. This function gives us the first indication of singular behavior, for it blows up when

$$t = t_c := \frac{1}{2f_1(f_1 - \epsilon)}\log\left(\frac{2f_1}{f_1 + \epsilon}\right) \sim \frac{\log(2)}{2f_1^2} \tag{A6}$$

To analyze the situation in more detail, we return to the full eq A3 and solve the characteristic equations

$$\frac{dt}{d\sigma} = 1 \Rightarrow t = \sigma + h_1(\tau)$$

$$\frac{dz}{d\sigma} = -2f_1(1 - e^{-z})(\epsilon - G_0(t)) \Rightarrow e^z = 1 + \exp(h_2(\tau) - 2f_1[\epsilon\sigma - \int_0^\sigma G_0(s)ds])$$

$$\frac{dF}{d\sigma} = 2f_1^2 e^{-z}(\epsilon - G_0(t)) \Rightarrow F = h_3(\tau) + 2f_1^2\int_0^\sigma \frac{[\epsilon - G_0(s)]ds}{1 + \exp[h_2(\tau) - 2f_1(\epsilon s - \int_0^s G_0(p)dp)]} \tag{A7}$$

Now we apply the initial conditions $F(z, 0) = f_1 e^{-z}$ on the line $\sigma = 0$

$$t = 0 \Rightarrow h_1(\tau) = 0$$
$$z = \tau \Rightarrow h_2(\tau) = \log(e^\tau - 1)$$
$$F = f_1 e^{-z} \Rightarrow h_3(\tau) = f_1 e^{-\tau} \tag{A8}$$

It is possible to eliminate both σ and τ to obtain the solution

$$F(z, t) = \frac{f_1}{1 + (e^z - 1)\exp(2f_1[\epsilon t - \int_0^t G_0(v)dv])} + \int_0^t \frac{2f_1^2[\epsilon - G_0(t - u)]du}{1 + (e^z - 1)\exp(2f_1[\epsilon u - \int_0^u G_0(t - p)dp])} \tag{A9}$$

however, it is not obvious from this how the individual concentrations $f_r(t)$ behave. We are in a similar position to that found in section 3.2, and so we again treat the chain length distribution function $f_r(t)/F(0, t)$ as a time-dependent probability distribution function, extracting the number of chains, expected length and standard deviations from the function $F(z, t)$.

The number density of chains,

$$N(t) = F(0, t) = f_1\left[1 + \log\left(\frac{(f_1 - \epsilon)}{2f_1 - (f_1 + \epsilon)e^{2tf_1(f_1-\epsilon)}}\right)\right] \tag{A10}$$

blows up at $t = t_c$ — the same place as $G_0(t)$ became singular

(eq A6). To perform a local analysis we put $t = t_c - \tau$; then $\mathbf{N} \sim -f_1 \log \tau$, as $\tau \to 0$. The expected chain length is

$$\mathbf{E}(t) = \frac{-1}{F(0, t)} \frac{\partial F}{\partial z}(0, t) =$$

$$\frac{\{(f_1 + \epsilon)e^{2tf_1(f_1 - \epsilon)} - 2\epsilon\}}{\{2f_1 - (f_1 + \epsilon)e^{2tf_1(f_1 - \epsilon)}\}} \frac{1}{\left[1 + \log\left(\dfrac{(f_1 - \epsilon)}{2f_1 - (f_1 + \epsilon)e^{2tf_1(f_1 - \epsilon)}}\right)\right]} \tag{A11}$$

which also blows up at $t = t_c$. Finally, the variance

$$\mathbf{V}(t) = \langle r^2 \rangle - \langle r \rangle^2 = \frac{1}{F(0, t)} \frac{\partial^2 F}{\partial z^2}(0, t) - \frac{G_0(t)^2}{F(0, t)^2} \tag{A12}$$

for general ϵ is a very complicated function, but in the limit $\epsilon \to 0$, it simplifies to

$$\mathbf{V}(t) = \frac{(2e^{2tf_1^2} - 1)[1 - \log(2 - e^{2tf_1^2})] - e^{4tf_1^2}}{(2 - e^{2tf_1^2})^2 [1 - \log(2 - e^{2tf_1^2})]^2} \tag{A13}$$

Plotting $\mathbf{E}$ and $(\mathbf{E} \pm \sqrt{\mathbf{V}})$ against time for $0 < t < t_c$, we find that the expected chain length ($\mathbf{E}$) remains small for a long period of time, before increasing rapidly as $t \to t_c$, where it diverges. $\mathbf{E} + \sqrt{\mathbf{V}}$ behaves similarly although it always lies above $\mathbf{E}$; however, $\mathbf{E} - \sqrt{\mathbf{V}}$ diverges to $-\infty$ as $t \to t_c$, implying that shorter chains are suppressed by the kinetics. These results suggest that uniform growth is only mildly unstable during periods of slow increase in concentrations of long RNA chains, but highly unstable when rapid change occurs.

References and Notes

(1) Eigen, M. *Die Naturwissenschaften* **1971**, *58*, 465.

(2) Gilbert, W. *Nature* **1986**, *319*, 618.

(3) Orgel, L. E. *Sci. Am.* **1994**, *271*, 77.

(4) Coveney, P. V.; Highfield, R. *Frontiers of Complexity*; Faber & Faber: London, 1995.

(5) Maynard Smith, J.; Szathmáry, E. *The Major Transitions in Evolution*; Oxford University Press: Oxford, 1997.

(6) Joyce, G. F. *Nature* **1989**, *338*, 217.

(7) Joyce, G. F.; Orgel, L. E. Prospects for understanding the origin of the RNA world; in *The RNA World*; Gesteland, R. F., Atkins, J. F., Eds.; Cold Spring Harbor Laboratory Press: New York, 1993; pp 1-26.

(8) Fleischaker, G.; Colonna, S.; Luisi, P. L. *Self-Production of Supramolecular Structures*; NATO ASI Series 446; Kluwer: Dordrecht, 1994.

(9) Eigen, M. *Steps Towards Life. A Perspective on Evolution*; Oxford University Press: Oxford, 1992.

(10) Eigen, M.; McCaskill, J.; Schuster, P. *J. Phys. Chem.* **1988**, *92*, 6881.

(11) Eigen, M.; McCaskill, J.; Schuster, P.; *Adv. Chem. Phys.* **1989**, *75*, 149.

(12) Eigen, M.; Schuster, P. *The Hypercycle. A Principle of Natural Self-Organisation*; Springer-Verlag: New York, 1979.

(13) Chacon, P.; Nuño, J. C. *J. Biol. Systems* **1995**, *3*, 351.

(14) Chacon, P.; Nuño, J. C. *Phys. D* **1995**, *81*, 398.

(15) Nuño, J. C.; Andrade, M. A.; Montero, F. *Bull. Math. Biol.* **1993**, *55*, 417.

(16) Nuño, J. C.; Andrade, M. A.; Moran, F.; Montero, F. *Bull. Math. Biol.* **1993**, *55*, 385.

(17) Nuño, J. C.; Montero, F.; de la Rubia, F. J. *J. Theor. Biol.* **1993**, *165*, 553.

(18) Nuño, J. C.; Tarazona, P. *Bull. Math. Biol.* **1994**, *56*, 875.

(19) Becker, R.; Döring, W. *Ann. Phys.* **1935**, *24*, 719.

(20) Penrose, O.; Lebowitz, J. L. In *Studies in Statistical Mechanics VII: Fluctuation Phenomena*; ed Montroll, E., Lebowitz, J. L., Ed.; North Holland: Amsterdam, 1976; pp 322−375.

(21) Coveney, P. V.; Wattis, J. A. D. *Proc. Roy. Soc. London, Ser. A* **1996**, *452*, 2079.

(22) Coveney, P. V.; Wattis, J. A. D. *J. Chem. Soc.: Faraday Trans.* **1998**, *94*, 233.

(23) Wattis, J. A. D.; Coveney, P. V. *J. Chem. Phys.* **1997**, *106*, 9122.

(24) Dold, J. W. *Proc. Roy. Soc. London, Ser. A* **1991**, *433*, 521.

(25) von Smoluchowski, M. *Phys. Z.* **1916**, *17*, 557.

(26) Ball, J. M.; Carr, J.; Penrose, O. *Commun. Math. Phys.* **1986**, *104*, 657.

(27) Carr, J. *Applications of Centre Manifold Theory*; Springer-Verlag: New York, 1981.

(28) Abramowitz, M.; Stegun, I. A. *Handbook of Mathematical Functions*; Dover: New York, 1972.

(29) Murray, J. D. *Biomathematics*; Springer-Verlag: Berlin, 1989; Vol. 19 (Mathematical Biology); Appendix 2.

(30) Falini, G.; Albeck, S.; Weiner, S.; Addadi, L. *Science* **1996**, *271*, 67.

(31) Kam, Z.; Shore, H. B.; Feher, G. *J. Mol. Biol.* **1978**, *123*, 539.

(32) Beckmann, W.; Otto, W. H. *Chem. Eng. Res. Des.* **1996**, *74*, 750.