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THEORETICAL PAPER

Models of Replicator Proliferation Involving Differential Replicator Subunit Stability

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Abstract

Several models for the origin of life involve molecules that are capable of self-replication, such as self-replicating polymers composed of RNA or DNA or amino acids. Here we consider a hypothetical replicator (AB) composed of two subunits, A and B. Programs written in Python and C programming languages were used to model AB replicator abundance as a function of cycles of replication (iterations), under specified hypothetical conditions. Two non-exclusive models describe how a reduced stability for B relative to A can have an advantage for replicator activity and/or evolution by generating free A subunits. In model 1, free A subunits associate with AB replicators to create AAB replicators with greater activity. In simulations, reduced stability of B was beneficial when the replication activity of AAB was greater than two times the replication activity of AB. In model 2, the free A subunit is inactive for some number of iterations before it re-creates the B subunit. A re-creates the B subunit with an equal chance of creating B or B′, where B′ is a mutant that increases AB' replicator activity relative to AB. In simulations, at moderate number of iterations (< 15), a shorter survival time for B is beneficial when the stability of B is greater than the inactive time of A. The results are consistent with the hypothesis that reduced stability for a replicator subunit can be advantageous under appropriate conditions.

Keywords Replicator · Subunit · Stability · Evolution · Complexity · Antagonistic pleiotropy

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Introduction

There is currently no universally accepted definition of life, however, most working definitions emphasize the ability to replicate (Cleland and Chyba 2002; Koshland 2002; Tsokolov 2009; McKay 2004). The cell is generally accepted to be the smallest unit of life, based upon its ability to replicate, as well as other properties, including the ability to store the information required to direct its own replication. In modern cells, this information is encoded in genes composed of DNA. The gene has historically been defined as the unit of heredity, and this definition has evolved as more is learned about the structure of genes (Portin and Wilkins 2017). One modern definition of the gene is "the unit of heredity—made of DNA or RNA , that encodes a coherent set of potentially overlapping functional product molecules, either protein or RNA —that influences phenotype in ways we may or may not be able to measure" (Hopkin 2009).

Hypotheses for the evolutionary origins of life often include early replicators that are composed of RNA or other polymers (Szilagyi et al. 2017; Stadler 2016; Czaran et al. 2015; Takeuchi and Hogeweg 2012; Banwell et al. 2018). In these models, the evolution of life proceeds through a stage where the gene is itself the replicator. One possibility is non-enzymatic replication, where the replicator polymer is not an enzyme, but instead acts as a scaffold to promote covalent attachments between activated monomers (Robertson and Joyce 2012; Szostak 2012). For example, an RNA primer annealed to an oligonucleotide with a poly-C templating region can be non-enzymatically elongated via primer-extension with appropriately activated G monomers (guanosine 5′-phosphor(2-methyl)imidazolide) (Adamala and Szostak 2013). Another possibility is enzymatic replication, where the replicator polymer is a polymerase enzyme capable of synthesizing new copies of itself, perhaps by using itself as a template for the polymerization reaction. Consistent with this idea, RNA molecules have been identified that catalyze a variety of reactions, including RNA polymerization (Horning and Joyce 2016; Robertson and Joyce 2014; Wochner et al. 2011). For example, screening of large libraries of random RNA sequences yielded the class I RNA ligase ribozyme, capable of catalyzing the ligation of two RNA molecules (Ekland et al. 1995). Further in vitro mutagenesis and selection yielded ribozymes that use an external RNA template and synthesize short strands of RNA by incorporating nucleoside triphosphates (Johnston et al. 2001). The crystal structure of the catalytic core suggests a mechanism wherein ribozyme phosphate groups bind a catalytic magnesium ion. The magnesium ion activates the 3'hydroxyl of the primer for nucleophilic attack with the incoming nucleoside triphosphate (Shechner and Bartel 2011). However, despite these and other successes, no single RNA molecule has yet been identified that can completely catalyze its own replication.

In modern cells, multiple genes cooperate to promote their own replication by encoding the components of the cell, including the multiple protein subunits of the chromosomal replisome (Yao and O'Donnell 2016). Similarly, several models for the evolution of early replicators involve cooperation between two or more polymeric species (Lincoln and Joyce 2009; Horning and Joyce 2016; Stadler 2016; Banwell et al. 2018; Wagner et al. 2017). One interesting question is how might the activity of a hypothetical multi-subunit replicator be affected by differences in the relative stability of the component subunits? The behavior of single-subunit replicators has been modelled using rate equations based on mass action kinetics (the replicator equation) (Stadler 2016), however, incorporating additional variables for subunit interactions and differential subunit stabilities would be computationally challenging. Here several simplifying assumptions were made to facilitate modeling of multi-subunit replicator activity using the Python and C programming languages.

In a general model for a two-subunit replicator, two subunits (A and B) bind to each other to produce an AB replicator, that in turn creates new copies of AB. One hypothesis is that if B is less stable than A (e.g., B has a shorter half-life than A), this can have an advantage for the replication activity and/or evolution of the AB replicator, by creating a more complex system (Fig. 1) (Tower 2006). If A and B are completely stable, then as a function of time this yields AB replicators (Fig. 1a). Similarly, if A and B are unstable to the same extent, for example, they have the same half-life or are degraded together, then as a function of time this yields AB replicators (Fig. 1b). In contrast, if B is less stable than A, then as a function of time this yields AB replicators and free A subunits (Fig. 1c). In this way, a reduced stability of B relative to A yields a more complex system (set {AB, A}) than is produced when A and B have the same stability (set {AB}). The more complex system (set {AB, A}) is hypothesized to have potentially advantageous properties that are not exhibited by the simpler system (set {AB}). Whereas increased complexity in terms of the number of distinct molecules is not necessarily advantageous, we suggest this increases the number of targets available for natural selection to create new and advantageous regulatory and catalytic interactions (Szathmary 2006; Takeuchi and Hogeweg 2008; Tower 2006); for example, the stimulatory effect of free A subunits on AB replicator activity proposed below in model 1. Here we further develop two non-exclusive models for how a reduced stability for B relative to A can be advantageous for AB replicator proliferation.

Methods

Computer programs were written in the C (Kernighan and Ritchie 1988) and Python (Python Software Foundation, Python Language Reference, version 3.6. Available at http://www.python.org) programming languages. The Numpy package of Python is required, therefore the Python distribution Anaconda is recommended, available at https://www.anaconda.com/download/. The software suite "Replication Program Model 1" (ESM_1.zip), contains the files used to model the stimulatory effect of free A subunits. "gene_1.exe" is the executable file that simulates the replication, and "gene_1.c" stores the primary code. "graph-drawing_1.py" is the Python executable file that yields graphs of the simulations. "in.txt" is the input file where parameters are set before running the simulation, and "out.txt" stores the results of the

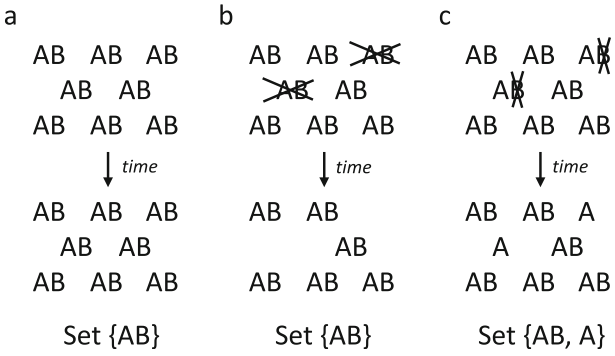

Fig. 1 Differential replicator subunit stability. A hypothetical replicator (AB) is considered, consisting of two subunits, A and B. **a** If A and B subunits are both completely stable, then as a function of time this yields AB replicators. **b** If A and B are both unstable to exactly the same extent, for example, they have the same half-life or are degraded together, then as a function of time this yields AB replicators. **c** If B is less stable than A, then as a function of time this yields AB replicators and free A subunits

simulation. The file "UserManualModel 1.pdf" is a detailed user manual for all software for model 1. The software suite "Replication Program Model 2" (ESM_2.zip), contains the files used to model the re-generation of B or B′ subunits. "GeneObject_2.py" is the Python file that builds up different genes as Python objects. "RecordResult_2.py" is the python file that simulates the replication. "GraphDrawing_2.py" is the file that yields graphs of the simulations. "GraphDrawing_2.py" is the only file that is needed to execute; it will automatically read from the other two files and produce the graphs. The file "UserManualModel 2.pdf" is a detailed user manual for all software for model 2.

Results

A hypothetical replicator (AB) is considered, that is composed of two subunits (A and B), and that catalyzes the creation of new AB replicators. Substrate is assumed to be non-limiting, and product inhibition is assumed to be absent. The stability of B and the concentration of AB replicators is expressed in terms of cycles of replication (iterations).

Model 1 involves a stimulatory effect of free A subunits (Fig. 2). The AB replicator replicates by converting an undefined number (n) of substrate molecules (S) into a new copy of AB (Fig. 2a). If the activity of the AB replicator is set to 1 (AB = 1), this means AB creates one new copy of AB at each iteration (Fig. 2a). If both subunits A and B are hypothesized to be stable, the AB replicator increases in concentration exponentially with each iteration (Fig. 2a; plot shown in Fig. 3a). If the B subunit has a reduced stability relative to A, then as a function of time B will be lost, yielding free A subunits (Fig. 1c). In this way, the instability of B will limit the exponential increase in AB concentration with time, because A cannot act by itself to create new AB replicators. Therefore, B's instability limits AB replicator proliferation, because loss of B inactivates the replicator. However, if there is also a stimulatory effect of free A subunits, this can potentially outweigh the inherent cost of B instability. In this model, free A subunits can associate with AB replicators to create AAB replicators (Fig. 2b). The AAB replicator can create new copies of AB, and potentially has increased activity relative to AB alone (Fig. 2b-d). The program "gene_1.c" (ESM_1.zip) was created using C programming language to model replicator activity under these conditions. Inputs included the stability of B (expressed in iterations), the replication activity of AB, the replication activity of AAB, and the number of iterations to be modeled.

Each set of conditions for stability of B is compared to the "baseline" condition, where B is completely stable and the replication activity of AB is set to 1, yielding an exponential increase in AB abundance (Fig. 3a, blue dashed line, indicated with asterisk; this simulation corresponds to the conditions and equation shown in Fig. 2a). We next simulated conditions where free A subunits combine with AB replicators to create AAB replicators, with potentially increased activity (Fig. 3b-d). When the replication activity of AAB is equal to the replication activity of AB (i.e., AB = 1, AAB = 1), then there is no stimulation by the free A, and the reduced stability of B is detrimental (Fig. 3b; these simulations correspond to the conditions and equations shown in Fig. 2b). Data is shown for 10 different simulations (Fig. 3b, solid lines) where the stability of B is varied from most stable (B = 10, i.e., B is stable for all 10 iterations, solid blue line), to least stable (B = 1, i.e., B is lost at each iteration, solid orange line). When B is completely stable, the simulation overlaps the baseline (indicated by asterisk). As the stability of B is progressively decreased, the simulations show progressively decreased replicator proliferation, moving in the direction of the black arrow, shown to the right of the plot. Therefore, under these conditions, the reduced stability of B causes a

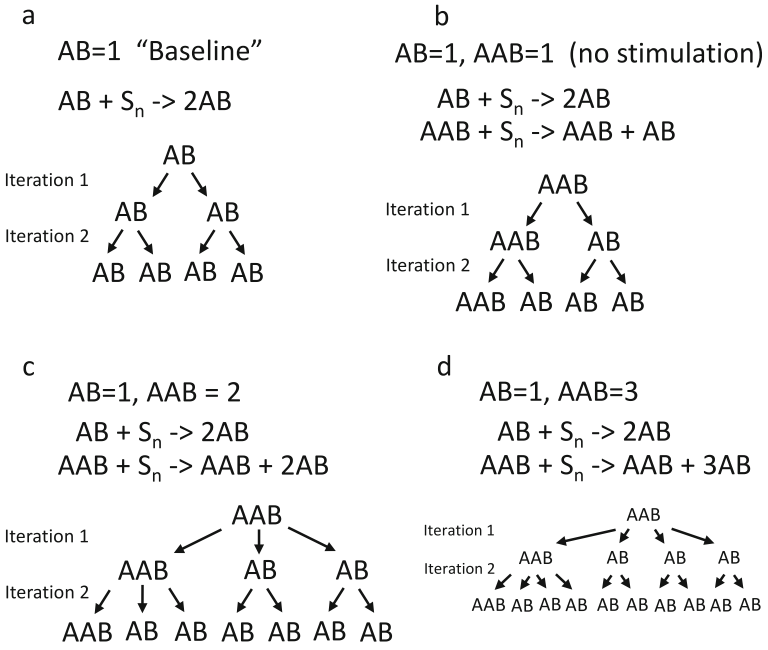

Fig. 2 Model 1 for stimulatory effect of free A subunits. **a** (AB = 1), baseline exponential replication by the AB replicator. In the control (or "baseline") condition, the AB replicator is assumed to be stable with time. In each cycle (or "iteration") of replication, the AB replicator creates one new copy of AB. In the equation presented, one AB replicator plus an undefined number (n) of substrate molecules (S) yields two copies of AB. (b-d) Under conditions where the B subunit is hypothesized to have limited stability this yields free A subunits, as shown in Fig. 1. In the simulations, the stability of B is defined in terms of iterations. Free A subunits combine with AB replicators to create AAB replicators; this association is assumed to be instantaneous and stable. Both the AB replicator and the AAB replicator create new copies of AB. **b** (AB = 1, AAB = 1). The replication activity of AAB is set to 1, such that in each iteration AAB creates 1 new copy of AB (AAB = 1). Under these conditions the association of A with AB to create AAB has no stimulatory effect on replication relative to the activity of AB. In the equation, one copy of AAB plus an undefined number (n) of substrate molecules (S) yields one copy of AAB and one copy of AB. **c** (AB = 1, AAB = 2) The replication activity of AAB is set to 2, such that in each iteration AAB creates 2 new copies of AB. Under these conditions the association of A with AB to create AAB has doubled the replication activity per iteration. In the equation, one copy of AAB plus an undefined number (n) of substrate molecules (S) yields one copy of AAB and two copies of AB. **d** (AB = 1, AAB = 3). The replication activity of AAB is set to 3, such that in each iteration AAB creates 3 new copies of AB. Under these conditions the association of A with AB to create AAB has tripled the replication activity per iteration. In the equation, one copy of AAB plus an undefined number (n) of substrate molecules (S) yields one copy of AAB and three copies of AB

progressive decrease in replicator proliferation (black arrow), below that of the baseline (asterisk), as expected (Fig. 3b). Similarly, when AB = 1, and AAB = 2, the activity of AAB is only two times that of AB. Under these conditions the reduced stability of B again causes a progressive decrease in replicator proliferation, below that of the baseline (Fig. 3c, solid lines; these simulations correspond to the conditions and equations shown in Fig. 2c). However, note that the decrease in replicator proliferation caused by B instability is less severe than when there was no stimulation by free A (compare Fig. 3c to b). In contrast, when the replication activity of AAB is three times the replication activity of AB (AB = 1, AAB = 3), then the reduced stability of B becomes beneficial (Fig. 3d, solid lines; these simulations correspond to the conditions and equations shown in Fig. 2d). Under these conditions the reduced stability of B causes a progressive increase in replicator proliferation (black arrow), above the baseline (asterisk; Fig. 3d). In conclusion, when the activity

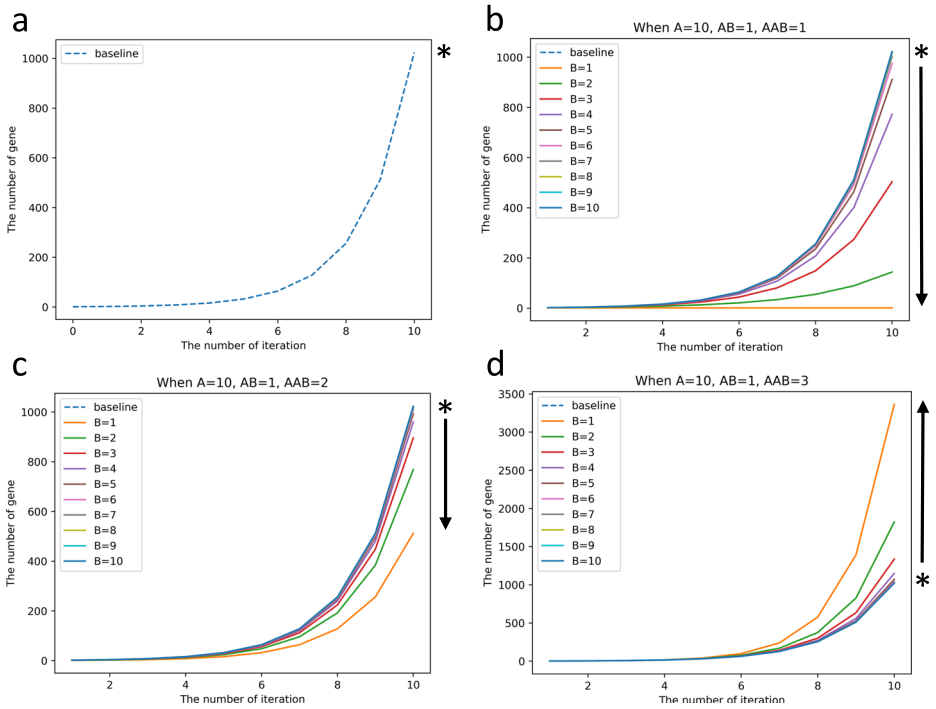

Fig. 3 Results of simulations for model 1 involving stimulatory effect of free A subunits. In each panel the X axis is the number of iterations, and the Y axis is the total number of AB replicators ("the number of gene"). **a** "Baseline" exponential replication by the AB replicator (AB = 1)(blue dashed line, indicated by asterisk). In the baseline condition, the AB replicator is assumed to be stable with time. The replication activity of AB is set to 1, such that in each iteration AB creates one new copy of itself. **b-d** Results of simulations where B has limited stability. Stability of A is set to 10 such that A is completely stable for the 10 iterations presented. The "baseline" (from a) is included in each panel for comparison (blue dashed line, indicated by asterisk). Results are presented for 10 different simulations in each panel (solid lines), where the stability of B varies from 1 iteration (B = 1, least stable) to 10 iterations (B = 10, completely stable). The arrow to the right of each panel indicates the change in replicator activity away from the baseline, as the stability of B is varied from 1 iteration (B = 1, least stable) to 10 iterations (B = 10, completely stable). **b** AB = 1, AAB = 1 (no stimulation). **c** AB = 1, AAB = 2 (two-fold stimulation). **d** AB = 1, AAB = 3 (three-fold stimulation)

of AAB is less than or equal to two times AB (Fig. 3b, c), then a reduced stability for B is detrimental, whereas when the activity of AAB is equal to (or greater than; data not shown) three times AB, then a reduced stability for B becomes beneficial (Fig. 3d).

The model 2 involves the replacement of lost B subunits with either a new B subunit or a mutant B′ subunit with greater activity (Fig. 4). Free A subunits are hypothesized to be incapable of self-replication, but are able to regenerate either a new B subunit or a mutant subunit B′. A does this by converting some undefined number of substrate molecules into the new copy of B or B′ ($A + S_n$ -> AB'); one hypothetical molecular mechanism for this activity is presented below (Fig. 6d). The "hibernation time of A" represents how long (measured in iterations) it takes for A to generate either B or B′. The resulting AB' replicators are assumed to have greater replication activity than do AB replicators. The program "GraphDrawing_2.py" (ESM_2.zip) models replicator activity under these conditions. Inputs include the stability of B (expressed in iterations), the hibernation time of A (expressed in iterations, i.e., how many iterations before A re-creates either B or B′), the ratio of AB:AB' as generated by free A subunits, the replication activity of AB, the replication activity of AAB, and the number of

iterations to be modeled (see Fig. 4a). Each set of conditions is compared to the "baseline" condition described above, where B is completely stable and AB = 1, as indicated by the blue dashed line and asterisk (Fig. 5). For the present set of simulations the ratio of AB:AB' was set to 1.0. The random() function in Python is used for producing AB and AB' corresponding to the chosen ratio. This function applies the Mersenne Twister mechanism (Matsumoto and Nishimura 1998) to generate a random float number uniformly in the semi-open range [0.0, 1.0) each time. If the random float number is no larger than 1-(1/(1 + ratio)), the program produces an AB, otherwise the program produces AB'. Note that these conditions result in a stochastic variation in the concentration of AB as a function of iterations. For this reason, the model is run 10 times for each condition to account for this stochastic variation.

In model 2, under conditions where B is unstable, the ratio of new B:B′ equals 1.0, and replication activity of AB' is greater than that of AB, logical deduction leads to the conclusion that the simulations will always exceed the baseline after sufficient iterations. The logic for this conclusion is that because the replication activity of AB' is larger than the replication activity of AB by at least 1, the number of new AB' replicators generated at each iteration is equal to or greater than the total number of new AB replicators generated. When the AB' replicators turn into free A subunits, and these free A subunits turn into AB or AB' replicators after sufficient iterations, this will cause the total number of AB' replicators to be larger than the total number of AB replicators. This deduction always follows as long as free A units have a finite chance to become AB'. For example, when B = 4, hibernation = 2, AB = 1, AB' = 2, and ratio = 1.0, then

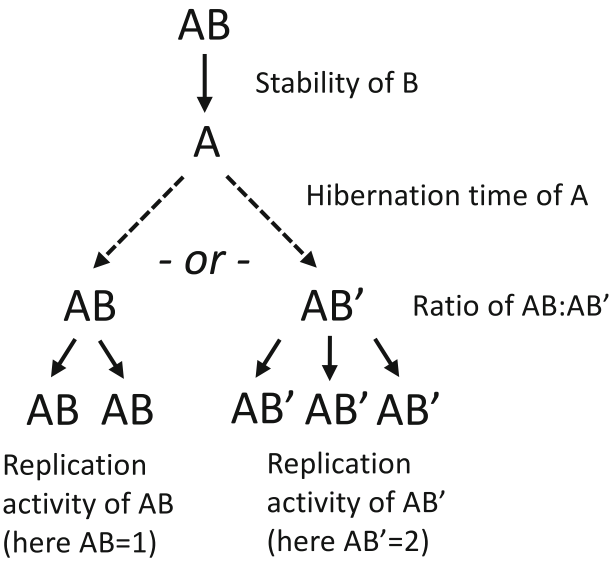

Fig. 4 Model 2 for creation of B′ subunit with increased replication activity.a Summary diagram for model 2. The model 2 includes 5 variables, indicated in small font. The program also takes an input for the number of iterations to be modeled, and the output is expressed as the total number of AB plus AB' replicators at each iteration. Stability of B is expressed as the number of iterations before B is lost. Hibernation time of A is the number iterations that A remains free, before A re-creates either a new B subunit or a new B′ subunit. Ratio of AB:AB' indicates the tendency of A to create new B subunits relative to new B′ subunits. Replication activity of AB is the number of new AB replicators that AB makes in each iteration, here shown as 1 (AB = 1, baseline condition). Replication activity of AB' is the number of new AB' replicators that AB' makes in each iteration, here shown as 2 (AB = 2)

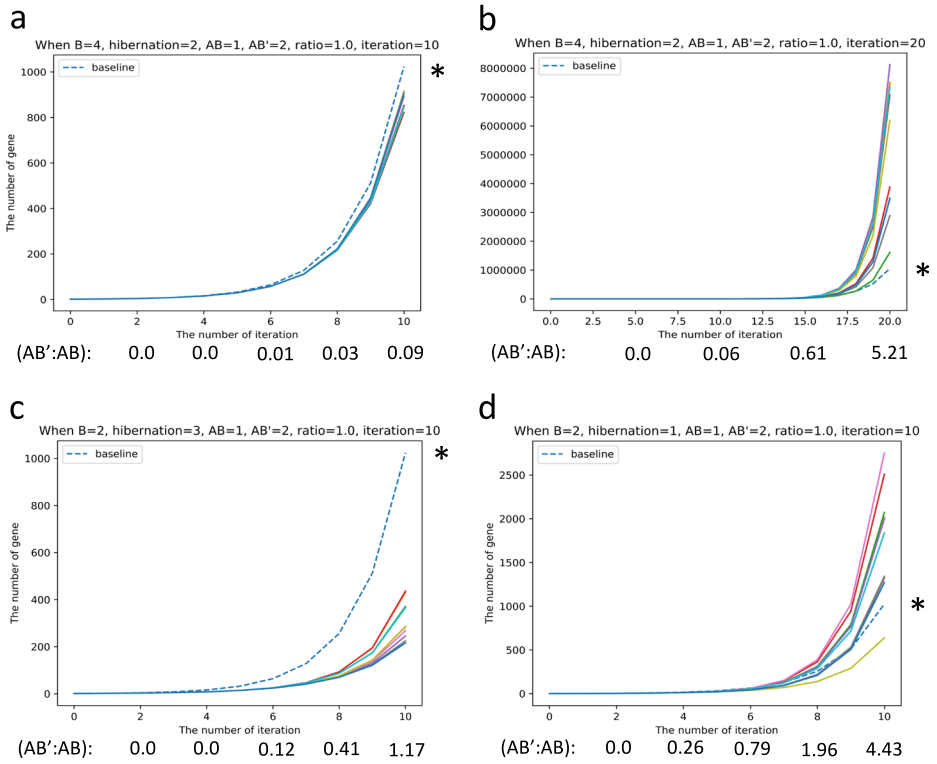

Fig. 5 Results of simulations for model 2 involving creation of B′. In each panel the X axis is the number of iterations, and the Y axis is the total number of AB replicators plus AB' replicators ("the number of gene"). **a-d** In each panel the results of 10 simulation runs are presented (solid lines), for the indicated conditions. The X axis is the iteration number. The Y axis is the number of genes (AB plus AB'). The average ratio of AB' to AB replicators at various iterations is presented below the X axis. The baseline condition (blue dashed line, indicated by asterisk) has been added to each panel to allow for comparisons. The baseline is as in previous figures, and indicates conditions where B is completely stable and AB = 1. **a** B = 4, hibernation = 2, AB = 1, AB' = 2, ratio = 1.0, iterations = 10. **b** B = 4, hibernation = 2, AB = 1, AB' = 2, ratio = 1.0, iterations = 20. **c** B = 2, hibernation = 3, AB = 1, AB' = 2, ratio = 1.0, iterations = 10. **d** B = 2, hibernation = 1, AB = 1, AB' = 2, ratio = 1.0, iterations = 10

by 10 iterations all ten simulations are below the baseline (Fig. 5a). However, under these same conditions, by iteration 20, all ten simulations now greatly exceed the baseline (Fig. 5b).

Decreasing the hibernation time for A results in the simulations exceeding the baseline at a smaller number of iterations. For example, when B = 2, hibernation = 3, AB = 1, AB' = 2, and ratio = 1.0, at iteration 10 all of the simulations are well below the baseline (Fig. 5c). In contrast, when all the conditions are the same, but hibernation time for A is reduced to 1, the simulations now overlap and often greatly exceed the baseline at iteration 10 (Fig. 5d). These results indicate that, under appropriate conditions, the limited stability of B in model 2 can result in a competitive advantage for replicator activity relative to the baseline, even at moderate numbers of iterations. Comparing the results of model 2 simulations under a variety of combinations of variables indicates that reducing the survival time for B is beneficial at moderate number iterations (< 15) when the stability of B is greater than the hibernation time of A (compare Fig. 5d to c, and additional data not shown).

In summary, the data indicate that for model 2, a finite stability of B is beneficial under most conditions after a sufficiently large number of iterations. At moderate numbers of iterations (<

15), a shorter survival time for B is beneficial when the stability of B is greater than the hibernation time of A.

Discussion

Here computer simulations were used to model the abundance of a hypothetical, two-subunit replicator (AB), as a function of cycles of replication (iterations). The results are consistent with the hypothesis that a reduced stability for subunit B relative to subunit A can be beneficial under appropriate conditions. In the first of two non-exclusive models (model 1), the limited stability of B was beneficial when the resultant free A subunits had a stimulatory effect on AB replicators. In model 2, the limited stability of B was beneficial when B was sometimes replaced by the more active mutant form B′.

One simplifying assumption made here is that iterations are used in place of time to define both replicator activity and replicator subunit stabilities. Additional simplifying assumptions include unlimited substrate availability, the absence of product inhibition, and instantaneous association of subunits. In the future, it may be of interest to attempt to apply rate equations, and to include these variables, to better match the expected natural conditions. Reaction-diffusion equations, including the Gray-Scott reaction-diffusion equation, model the concentration of molecules in space and time using variables representing chemical reactions and the diffusion of molecules (Gray and Scott 1985; Pearson 1993; Kosikova and Philp 2017; McCaskill 2018). These equations can include variables for the stability or transformation of molecules, as well as stimulatory and inhibitory interactions. In the future, it may be of interest to use reaction-diffusion equations to model aspects of replicator proliferation involving differential replicator subunit stability, such as the stability of B subunits and the diffusion of free A subunits.

Modeling in terms of iterations is certainly an over-simplification relative to the expected natural conditions, however, this choice does not seem unreasonable given the cyclical nature of replicators and their proposed evolution. Conceivably, iterations could be related to natural environmental cycles, such as hot/cold, light/dark, hydration/desiccation or freeze/thaw. For example, cyclical variations in temperature might favor an alternation between polymerization and product release, such as in PCR. Interestingly, a water ice environment and freeze/thaw cycles have been shown to favor several aspects of RNA polymerase ribozyme function (Attwater et al. 2013; Attwater et al. 2010; Mutschler et al. 2015). Virtually all cells exhibit circadian and/or other rhythms in metabolism and replication (e.g., the cell cycle), consistent with an important role for environmental cycles in the evolution of modern replicators (Dunlap and Loros 2017; Paranjpe and Sharma 2005; Dvornyk et al. 2003; Wagner et al. 2015).

The evolutionary fitness of a replicator can be generally described as its reproductive success, and this will be affected by multiple variables, including replicator activity, replicator stability, and competition with other replicators for substrate. In the present models, decreased stability of subunit B directly reduces AB replicator activity, because subunit A cannot self-replicate. During replicator evolution, decreased replicator activity is not expected to be the direct target of selection, as decreased replicator activity is expected to be inherently detrimental to fitness. However, selection for increased replicator activity through the stimulatory effect of free A subunits (model 1), or through the increased activity of B′ (model 2), might result in decreased stability of B as a consequence or trade-off. In these models, the gene encoding subunit B may be thought of as exhibiting antagonistic pleiotropy (Williams 1957), because B activates replicator activity in one context and inhibits replicator activity in another context. B is

an activator, because B is essential for the self-replication activity of the AB replicator. B is also an inhibitor, because B sequesters A subunits; these A subunits might otherwise be able to create more active AAB replicators (model 1) or AB' replicators (model 2). Because of this antagonistic pleiotropy of B functions, the trait of being unstable is detrimental to replicator activity in one context and is favorable to replicator activity in another context.

It is perhaps not surprising that we were able to model conditions where the limited stability of B was beneficial, given that these models were specifically contrived to create such a benefit. However, we suggest that because a beneficial effect of limited replicator subunit stability was readily obtained suggests that these models warrant further investigation, and may be relevant to replicator evolution. Many models of replicator evolution involve the creation of mutant replicators with increased activity as part of the normal replication cycles. In model 2, the generation of B′ is dependent upon the loss of B. This is because the chances of creating B′ through its replacement (which are finite in model 2) are different than the chances of its being created through self-replication of AB (which is assumed to be zero in the current model 2). This difference is hypothesized to be possible because the mechanism for B replacement by A is by definition different than the mechanism for self-replication by AB. One possible molecular interpretation of these models involves an RNA polymerase ribozyme (Fig. 6). Many different variations on these models appear possible, including both enzymatic and non-enzymatic

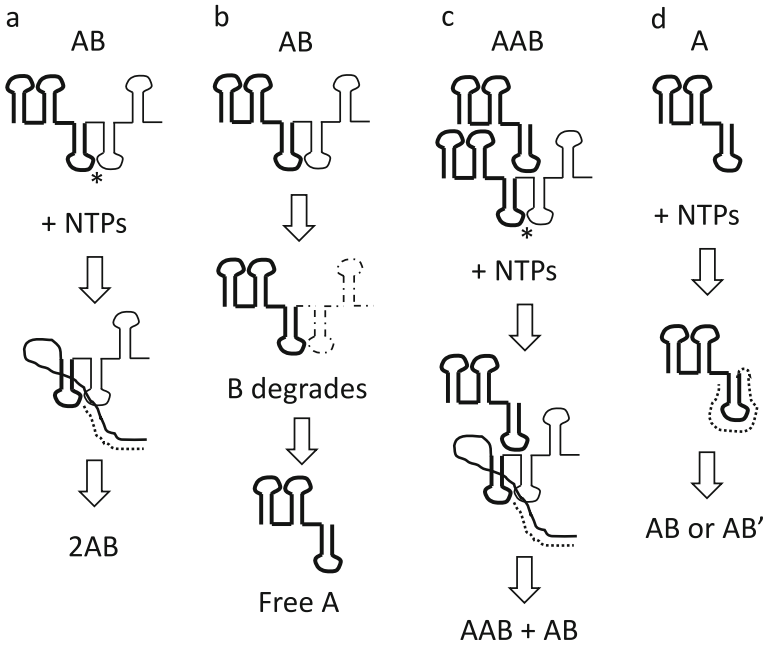

Fig. 6 One possible molecular interpretation of the replicator models. The AB replicator is diagrammed, with subunit A indicated in heavy line, and subunit B indicated in thin line. Here AB is a linear RNA polymer that folds into a 3D structure that generates an RNA polymerase enzyme active site (indicated by asterisk). **a** AB replicates by using various regions of itself as a template. Dotted line indicates nascent RNA. **b** Instability of B. Loss of B might occur through hydrolysis that proceeds from the end of the polymer. Alternatively, an enzyme might catalyze degradation of B from the end of the polymer. Free A subunit is hypothesized to be unable to efficiently self-replicate. **c** AAB replicators. In model 1, the free A subunits are hypothesized to associate with AB replicators to create AAB replicators, with potentially greater activity. **d** In model 2, the free A subunit is again hypothesized to be unable to self-replicate, but does retain sufficient RNA polymerase activity to re-create B or B′ by polymerizing an extension of its end, and using itself as a template (extension indicated by dotted line)

replication, and may be of interest to pursue in the future. In the present simulations, A is set to be completely stable (model 1), and the ratio of newly synthesized B:B′ is set to 1.0 (model 2), however the current software allows these values to be adjusted, and it may be of interest in the future to examine the effects of altering these variables. Moreover, by modifying the software, it should be possible to add additional species such as ABB replicators, and to include stimulatory and inhibitory effects of free B subunits, etc. Depending on the number of variables chosen, it may be possible to further automate how the software compares combinations of variables. One possibility might be to try incorporating differential replicator subunit stability into the hypercycle model (Eigen and Schuster 1978; Szostak et al. 2016).

In modern cells, there are several examples where degradation of one subunit in a multi-protein complex regulates the replication of cells and/or DNA. For example, in eukaryotes, cyclin proteins form complexes with cyclin-dependent kinases (CDKs), and cyclin degradation is required for progression through the stages of the cell cycle (Heim et al. 2017). Also in eukaryotes, DNA polymerase δ4 consists of 4 subunits and catalyzes the synthesis and processing of Okazaki fragments at the lagging strand. In response to DNA damage, the smallest subunit of DNA polymerase δ4 is degraded, yielding DNA polymerase δ3 with altered processivity and substrate specificity for DNA repair (Lee et al. 2014). In the future, it may be of interest to test whether incorporating a nuclease with appropriate activity and substrate specificity might increase the fitness of a multi-subunit RNA replicator or RNA network in vitro. It might also be of interest to explore whether replicators with differential subunit stability might be employed to increase the variety of structures that can be created through self-assembly (Ke et al. 2018; Okesola and Mata 2018; Altay et al. 2017)

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References

Adamala K, Szostak JW (2013) Nonenzymatic template-directed RNA synthesis inside model protocells. Science 342(6162):1098–1100

Altay Y, Tezcan M, Otto S (2017) Emergence of a new self-replicator from a dynamic combinatorial library requires a specific pre-existing replicator. J Am Chem Soc 139(39):13612–13615

Attwater J, Wochner A, Pinheiro VB, Coulson A, Holliger P (2010) Ice as a protocellular medium for RNA replication. Nat Commun 1:76

Attwater J, Wochner A, Holliger P (2013) In-ice evolution of RNA polymerase ribozyme activity. Nat Chem 5(12):1011–1018

Banwell EF, Piette B, Taormina A, Heddle JG (2018) Reciprocal Nucleopeptides as the ancestral Darwinian self-replicator. Mol Biol Evol 35(2):404–416

Cleland CE, Chyba CF (2002) Defining 'life. Orig Life Evol Biosph 32(4):387–393

Czaran T, Konnyu B, Szathmary E (2015) Metabolically coupled replicator systems: overview of an RNA-world model concept of prebiotic evolution on mineral surfaces. J Theor Biol 381:39–54

Dunlap JC, Loros JJ (2017) Making time: conservation of biological clocks from fungi to animals. Microbiol Spectr 5(3). https://doi.org/10.1128/microbiolspec.FUNK-0039-2016

Dvornyk V, Vinogradova O, Nevo E (2003) Origin and evolution of circadian clock genes in prokaryotes. Proc Natl Acad Sci U S A 100(5):2495–2500

Eigen M, Schuster P (1978) The Hypercycle. Naturwissenschaften 65(1):7–41

Ekland EH, Szostak JW, Bartel DP (1995) Structurally complex and highly active RNA ligases derived from random RNA sequences. Science 269(5222):364–370

Gray P, Scott SK (1985) Sustained oscillations and other exotic patterns of behavior in isothermal reactions. J Phys Chem 89:22–32

Heim A, Rymarczyk B, Mayer TU (2017) Regulation of cell division. Adv Exp Med Biol 953:83–116

Hopkin K (2009) The evolving definition of a gene. BIoScience 59(11):928–931
Horning DP, Joyce GF (2016) Amplification of RNA by an RNA polymerase ribozyme. Proc Natl Acad Sci U S A 113(35):9786–9791
Johnston WK, Unrau PJ, Lawrence MS, Glasner ME, Bartel DP (2001) RNA-catalyzed RNA polymerization: accurate and general RNA-templated primer extension. Science 292(5520):1319–1325
Ke Y, Castro C, Choi JH (2018) Structural DNA nanotechnology: artificial nanostructures for biomedical research. Annu Rev Biomed Eng 20:375–401
Kernighan BW, Ritchie DM (1988) *The C programming language*. Prentice Hall, Englewood Cliffs
Koshland DE Jr (2002) Special essay. The seven pillars of life. Science 295(5563):2215–2216
Kosikova T, Philp D (2017) Exploring the emergence of complexity using synthetic replicators. Chem Soc Rev 46(23):7274–7305
Lee MY, Zhang S, Lin SH, Wang X, Darzynkiewicz Z et al (2014) The tail that wags the dog: p12, the smallest subunit of DNA polymerase delta, is degraded by ubiquitin ligases in response to DNA damage and during cell cycle progression. Cell Cycle 13(1):23–31
Lincoln TA, Joyce GF (2009) Self-sustained replication of an RNA enzyme. Science 323(5918):1229–1232. https://doi.org/10.1126/science.1167856
Matsumoto M, Nishimura T (1998) Mersenne twister: a 623-dimensionally equidistributed uniform pseudo-random number generator. ACM Trans Model Comput Simul 8(1):3–10
McCaskill JS (2018) From quasispecies to quasispaces: coding and cooperation in chemical and electronic systems. Eur Biophys J 47(4):459–478
McKay CP (2004) What is life–and how do we search for it in other worlds? PLoS Biol 2(9):E302
Mutschler H, Wochner A, Holliger P (2015) Freeze-thaw cycles as drivers of complex ribozyme assembly. Nat Chem 7(6):502–508
Okesola BO, Mata A (2018) Multicomponent self-assembly as a tool to harness new properties from peptides and proteins in material design. Chem Soc Rev 47:3721–3736
Paranjpe DA, Sharma VK (2005) Evolution of temporal order in living organisms. J Circadian Rhythms 3(1):7
Pearson JE (1993) Complex patterns in a simple system. Science 261(5118):189–192
Portin P, Wilkins A (2017) The evolving definition of the term "gene". Genetics 205(4):1353–1364
Robertson MP, Joyce GF (2012) The origins of the RNA world. Cold Spring Harb Perspect Biol 4(5):a003608. https://doi.org/10.1101/cshperspect.a003608
Robertson MP, Joyce GF (2014) Highly efficient self-replicating RNA enzymes. Chem Biol 21(2):238–245
Shechner DM, Bartel DP (2011) The structural basis of RNA-catalyzed RNA polymerization. Nat Struct Mol Biol 18(9):1036–1042
Stadler PF (2016) Evolution of RNA-based networks. Curr Top Microbiol Immunol 392:43–59
Szathmary E (2006) The origin of replicators and reproducers. Philos Trans R Soc Lond Ser B Biol Sci 361(1474):1761–1776
Szilagyi A, Zachar I, Scheuring I, Kun A, Konnyu B et al (2017) Ecology and evolution in the RNA world dynamics and stability of prebiotic replicator systems. Life (Basel) 7(4):E48. https://doi.org/10.3390/life7040048
Szostak JW (2012) The eightfold path to non-enzymatic RNA replication. J Syst Chem 3:2
Szostak N, Wasik S, Blazewicz J (2016) Hypercycle. PLoS Comput Biol 12(4):e1004853
Takeuchi N, Hogeweg P (2008) Evolution of complexity in RNA-like replicator systems. Biol Direct 3:11
Takeuchi N, Hogeweg P (2012) Evolutionary dynamics of RNA-like replicator systems: a bioinformatic approach to the origin of life. Phys Life Rev 9(3):219–263
Tower J (2006) Sex-specific regulation of aging and apoptosis. Mech Ageing Dev 127(9):705–718
Tsokolov SA (2009) Why is the definition of life so elusive? Epistemological considerations. Astrobiology 9(4):401–412
Wagner N, Alasibi S, Peacock-Lopez E, Ashkenasy G (2015) Coupled oscillations and circadian rhythms in molecular replication networks. J Phys Chem Lett 6(1):60–65
Wagner N, Mukherjee R, Maity I, Peacock-Lopez E, Ashkenasy G (2017) Bistability and bifurcation in minimal self-replication and nonenzymatic catalytic networks. Chemphyschem 18(13):1842–1850
Williams GC (1957) Pleiotropy, natural selection, and the evolution of senescence. Evolution 11:398–411
Wochner A, Attwater J, Coulson A, Holliger P (2011) Ribozyme-catalyzed transcription of an active ribozyme. Science 332(6026):209–212
Yao N, O'Donnell M (2016) Bacterial and eukaryotic replisome machines. JSM Biochem Mol Biol 3(1):1013