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Minimal self-replicating systems

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Minimal self-replicating systems typically consist of three components: a product molecule, and two substrate molecules that become joined to form another product molecule. An important characteristic of self-replicating systems is the ability of the product to catalyze the formation of additional product, resulting in autocatalytic behavior. Recent advances in the area of self-replication have led to improved efficiency of autocatalysis, both by increasing the fraction of product molecules that can participate in further rounds of replication, and by improving the efficiency of the catalysts themselves. This review analyzes chemical self-replicating systems that have been developed to date and discusses ongoing challenges in this area of research.

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Abbreviations

DCC dicyclohexylcarbodiimide
DCU dicyclohexylurea

Introduction

Nature's ability to generate identical copies of the macromolecules necessary for life is a complex process, involving multiple reaction steps that must be tightly coordinated for efficient copying to occur. Much effort has gone into unraveling the intricacies of these biochemical events, which fundamentally entail a template molecule that directs the formation of product molecules through a series of reaction steps that are controlled by macromolecular catalysts. A simplified version of this system might employ a single template molecule that also functions as a catalyst. The system is considered to be self-replicating if the product, formed from two or more component substrates, is identical to the template. This review discusses such simplified systems for chemical self-replication and highlights the obstacles that must

be overcome to make these systems more efficient and more complex.

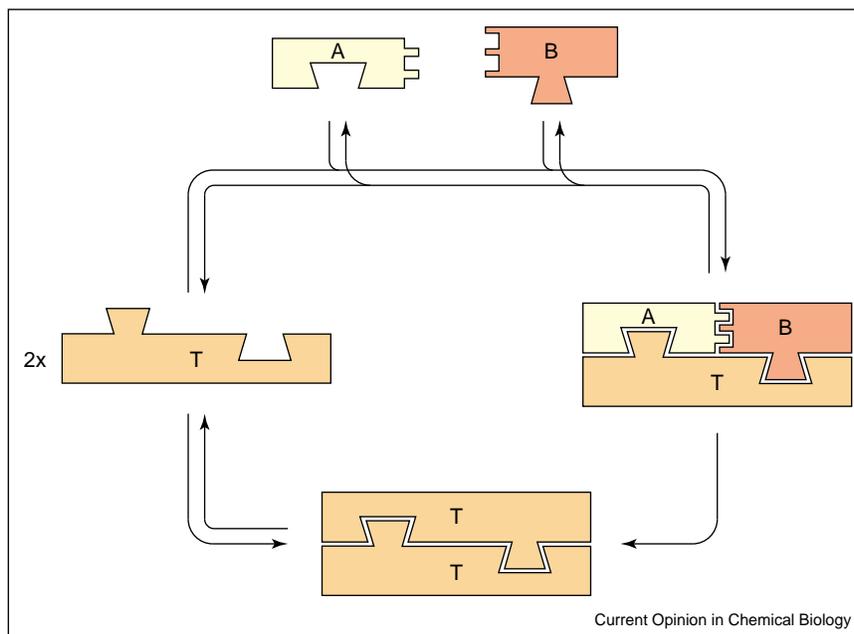
Minimal self-replicating systems are composed of two substrate molecules, **A** and **B**, and a template/product molecule, **T** (Figure 1) [1–4]. In each cycle of replication, **T** binds **A** and **B** to form a ternary complex (**A•B•T**), positioning the reactive ends of the two substrates in close proximity to facilitate covalent bond formation. The joining of **A** and **B** results in the formation of another copy of **T**. The template-product complex (**T•T**) must then dissociate to provide two free copies of **T**, which can enter the next cycle of replication. Many examples of minimal self-replicating systems have been demonstrated, involving nucleic acids [5–7,8**], peptides [9–12,13**,14*], and even small organic molecules [15–17].

Toward efficient self-replication

Although self-replicating systems are conceptually simple, there are several aspects of the chemistry that must be optimized to provide a system that exhibits efficient autocatalytic behavior. A first requirement is that the template molecule, **T**, substantially accelerates the rate of reaction between the substrates **A** and **B** compared with their rate of reaction in the absence of **T**. A common measure of the rate acceleration attributable to **T** is the autocatalytic efficiency, ϵ , which is the ratio of the template-catalyzed rate and the template-independent rate [7]. A second requirement is that the ternary complex, **A•B•T**, forms readily. The inherent symmetry of the system may lead to competing interactions, such as an **A•B** substrate complex, or template self-structure involving the two complementary substrate-recognition domains within **T**. A third requirement is that the release of the newly formed product occurs readily. A common measure of the dimerization state of the template-product complex is the order of the reaction, p . A value for p of 0.5 corresponds to rate-limiting dissociation, while a value of 1.0 corresponds to efficient dissociation allowing autocatalytic, exponential amplification [2,18]. To achieve a proper balance between effective binding of the substrates to the template and facile product release, the system often must be so highly optimized that it is intolerant of change. Several noteworthy advances have been reported over the past few years that address one or more of the requirements described above.

One well-studied class of self-replicating systems involves peptides that contain heptad repeats, where the first and fourth positions of the repeat are hydrophobic amino acids [9–13,14*,19]. Such sequences form α -helices, which can assemble into a coiled-coil structure

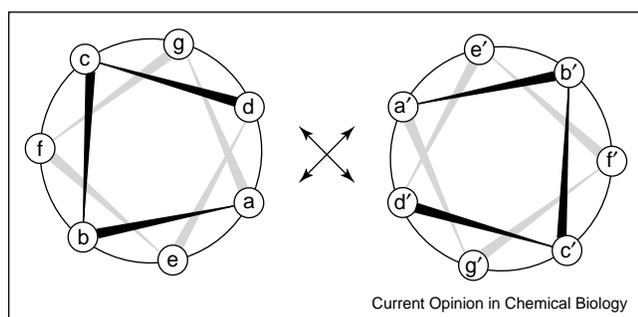
Figure 1



Minimal self-replicating system. The template (**T**) binds two substrates (**A** and **B**), which become joined to form another copy of **T**. Following dissociation of the template–product complex, each copy of **T** can enter another replication cycle.

through associations between the hydrophobic residues (Figure 2, Table 1). A full-length peptide template, **T**, directs the condensation of the two half-length peptide substrates, **A** and **B**, employing thioester-promoted native ligation chemistry to form an amide linkage between the two substrates [20]. Initial versions of this scheme employed templates containing either 32 or 35 amino acids, and exhibited a reaction order of 0.5 [9–12]. Such systems were limited by slow release of the newly formed product. Recent advances from the Chmielewski laboratory have implemented two strategies to achieve a reaction order approaching 1.0, which would be indicative of exponential amplification.

Figure 2



Helical wheel diagram for peptide replicators designed to fold into α -helices. Within the heptad repeat, the residues a, d, a', and d' engage in hydrophobic packing interactions, and the residues e, g, e' and g' engage in electrostatic interactions.

One strategy that resulted in improved peptide self-replication was to reduce the length of the template to 26 amino acids to destabilize the template–product complex [13**] (Table 1). This led to a reaction order of 0.91 and the highest autocatalytic efficiency ($\epsilon = 1.0 \times 10^5$) ever demonstrated for a peptide replicator. In shortening the peptide, the number of leucine residues was reduced and the overall hydrophobicity of the templating surface was decreased. This resulted in more efficient product release, therefore facilitating successive cycles of replication.

The second strategy for improving product release was to change a glutamate to a proline at the fifth position of the heptad repeat, with the aim of distorting the coiled-coil structure of the template–product complex [14*] (Table 1). This substitution introduced a 30° kink in the α -helix, leading to a reaction order of 0.91 and an autocatalytic efficiency of 3.2×10^4 . The position of the proline substitution, located on the hydrophilic face of the helix, was found to be critical for this improved behavior. The substitution did not disrupt the hydrophobic templating face, allowing efficient complexation between the template and substrates, while the kink resulted in improved dissociation of the template–product complex.

Initial examples of self-replicating systems composed of nucleic acids employed a template that formed a continuous stretch of base pairing with the two substrates [5–7]. This allowed for effective positioning of the reac-

Table 1

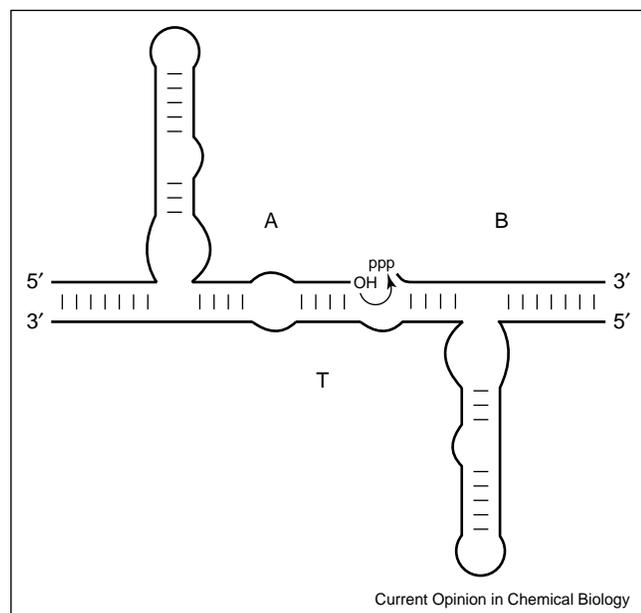
Autocatalytic efficiency (ϵ) and reaction order (ρ) of various self-replicating peptides.

Peptide sequence	Subunits	ϵ	ρ	Reference
g abcdefg abcdefg ab•cdefg abcdefg abcdef (C)				
R MKQLEEK VYELLSK VA•CLEYE VARLKKL VGE (C)	2	500	0.5	[9]
R MKQLEEK VYELLSK VA•CLEXE IARLKKL IGE (C)	2	470	0.5	[10]
E LYALEKE LGALEKE LA•CLEKE LGALEKE LYALEK (C)	2 or 3	900	0.5	[11]
K LYALKEK LGALKEK LA•CLKEK LGALKEK LYALKE (C)	4	280	0.5	[12]
LEKE LYALEKE LA•CLEKE LYALEKE L (C)	4	100 000	0.9	[13]
E LYALEKE LGALEKE LA•CLPKE LGALEKE LYALEK (C)	4	32 000	0.9	[14*]

(C) indicates carboxy terminus; dot indicates ligation junction.

tion components, but caused product release to be rate limiting, resulting in a reaction order of 0.5. A recent example of a self-replicating nucleic acid involved an RNA template that also had inherent catalytic activity (Figure 3) [8**]. The template was a ribozyme, derived from the R3C ligase ribozyme [21]. It catalyzed the joining of two RNA substrates to produce another copy of the template/ribozyme. The templating interactions involved five discontinuous regions of Watson–Crick base pairing. In the absence of preformed template, the substrates **A** and **B** reacted at a very slow rate, but in the presence of template the rate of the reaction was greatly enhanced, with an autocatalytic efficiency of 3.0×10^8 . An added benefit of this system is that the discontinuous templating interactions enabled rapid product release, analogous to the effect of the proline kink in the efficient peptide replicator. This resulted in a reaction order of 1.0.

Figure 3



Secondary structure of the self-replicating ligase ribozyme. The RNA backbone is indicated by thick lines. The curved arrow indicates the site of ligation between the two substrates (**A** and **B**) to form another copy of the template (**T**).

However, although the template–product complex dissociated readily, exponential amplification was limited by a tendency for the substrate molecules to form an **A•B** dimer and for the template to form an intramolecular self-structure.

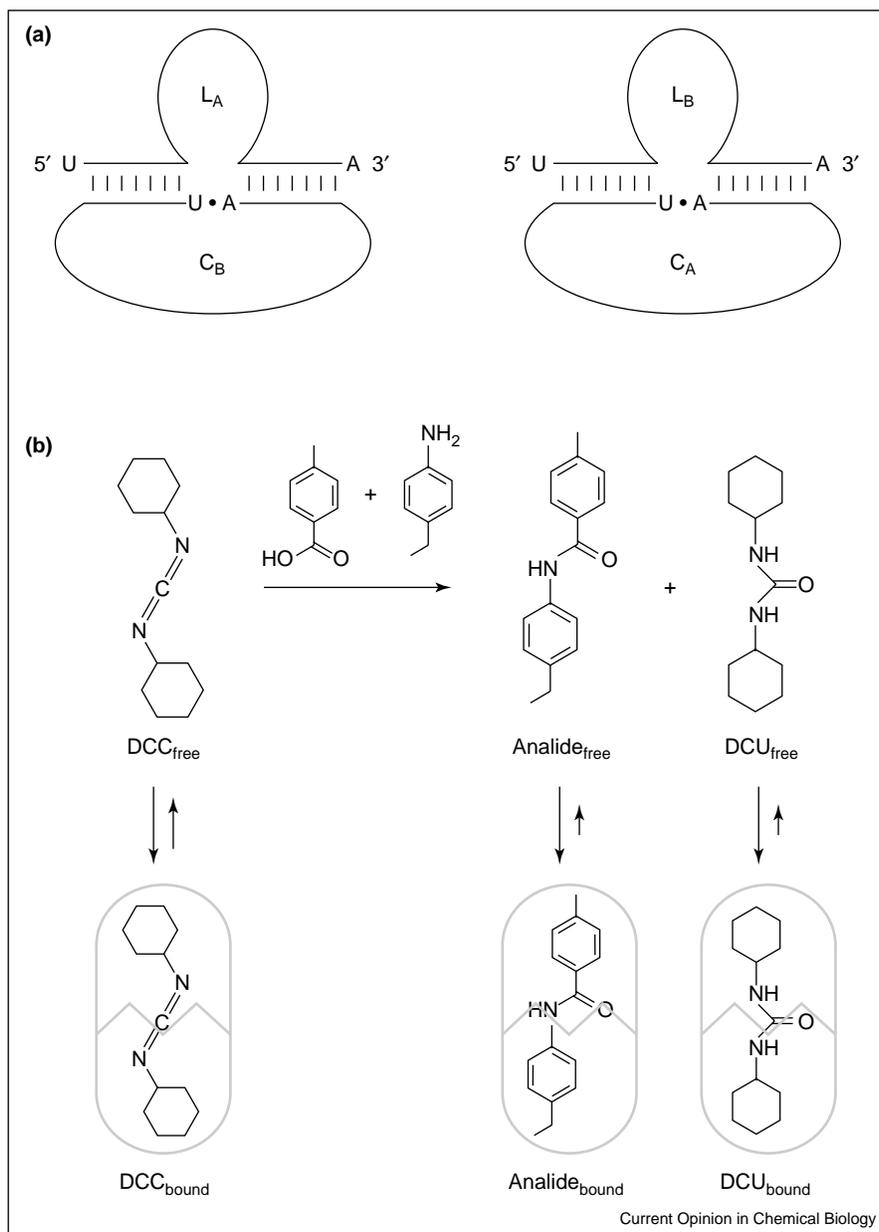
Significant advances have been made in the development of more efficient chemical self-replicating systems. By facilitating the dissociation of the template–product complex by various means, the autocatalytic efficiency of these systems has been improved substantially. Future advances will build upon this progress, striving for improved specificity of the template–substrate interactions relative to competing interactions, and for the sustained exponential amplification of the replicating species.

A broader notion of autocatalysis

Two recent reports in the area of chemical catalysis have broadened the notion of autocatalytic chemical processes. These are not self-replicating systems *per se* because they do not involve a joining reaction to form a product molecule that catalyzes its own formation. Rather, product formation is the result of a bond-breaking or molecular dissociation event, and it is this event that occurs autocatalytically.

One study involved a catalytic DNA molecule with RNA-cleavage activity [22]. Two different versions of the catalytic DNA were constructed, each specific for a different RNA substrate [23**]. Each version could exist in either an active linear form (L_A and L_B) or an inactive circular form (C_A and C_B) (Figure 4a). The circular form included an RNA substrate domain that, when cleaved, resulted in conversion of the circular form to the corresponding linear form. In this system, L_A catalyzes the cleavage of C_B to form L_B , while L_B catalyzes the cleavage of C_A to form L_A . As more copies of the linear molecules are produced, the rate of cleavage of the circular precursors increases. This behavior is best described as cross-catalytic rather than autocatalytic, because the two products catalyze each other's formation from the corresponding circular precursors [9,12,24]. Because the reaction involves cleavage rather than ligation, the product release step is not rate-limiting. As a result, the initial rate

Figure 4



Autocatalytic amplification systems that are not self-replicating. **(a)** Components of a cross-catalytic system based on two RNA-cleaving deoxyribozymes. Each of two circular precursors (C_A and C_B) contains a deoxyribozyme domain and a target RNA phosphodiester ($U \bullet A$). The deoxyribozyme is inactive until the circle is cleaved by the opposing linearized molecule: L_A cleaves C_B to form L_B ; L_B cleaves C_A to form L_A . **(b)** Autocatalysis through reagent compartmentalization. When DCC is released from the compartment, it reacts with the two substrates to form two products, each of which can occupy a compartment with greater affinity compared with DCC.

of product formation was found to increase linearly with increasing starting concentration of product, with a reaction order of 1.0 and an autocatalytic (cross-catalytic) efficiency of 1.2×10^9 . This is the highest autocatalytic efficiency reported to date.

A special feature of the study involving cross-catalytic DNA molecules was that *in vitro* selection experiments were used to identify preferred sequences within the

substrate-recognition domains of the catalytic DNAs. A pool of circular molecules was constructed that contained random-sequence nucleotides at these positions. The circular molecules were incubated under conditions that allowed some linear species to form. Then an aliquot of the reaction mixture, which contained both unreacted (circular) and reacted (linear) species, was transferred to a fresh reaction vessel that contained only unreacted molecules. The linear molecules that were carried over from

the previous reaction mixture had the opportunity to react with their cross-catalytic partners, leading to selective enrichment of the most active molecules. This process was repeated for four transfers, after which the linear molecules were isolated from the final population and sequenced. As expected, the 'winning' molecules had especially strong base-pairing potential (i.e. high G-C content), enabling tighter interaction with the circular precursors. This study provides a good example of how to conduct a thorough examination of sequence variants to isolate molecules with improved autocatalytic behavior.

Another study that has broadened the notion of autocatalysis implemented a reagent compartmentalization approach to chemical amplification [25•,26•]. A molecular 'host' was designed that encapsulates a precursor reagent, dicyclohexylcarbodiimide (DCC), rendering it unable to catalyze the reaction between the substrates *p*-toluic acid and *p*-ethylalanine (Figure 4b). Any molecules of DCC that escaped from the compartment could catalyze the reaction between the two substrates, resulting in formation of an analide product and dicyclohexylurea (DCU). For every DCC molecule that escaped the compartment, two product molecules were formed, each of which could occupy a free compartment with greater affinity compared with that of the DCC precursor. Although the product molecules did not catalyze the reaction between the two substrate molecules, a competitive equilibrium was established whereby the products facilitated the release of additional precursor molecules, thus causing the reaction to proceed in an autocatalytic manner.

Ongoing challenges

The development of an efficient self-replicating system requires a delicate balance between selective association and facile dissociation of the reaction components. Most of the templates that have been examined to date provide a surface that allows for hydrophobic and/or hydrogen-bonding interactions with the substrates. A notable advance would involve the discovery of a template that forms a ternary complex with the substrates via specific tertiary interactions that do not form between the substrates alone. Once the ternary complex has formed and the joining reaction has occurred, efficient template-product dissociation also must be achieved. Thus far, most solutions for facilitated product release have involved templates with either shortened substrate-recognition domains or discontinuous substrate-binding interactions. Alternatively, it would be advantageous to develop a template that undergoes a conformational change following the joining reaction, thereby facilitating product release without impairing substrate binding.

Once the framework for an efficient, minimal self-replicating system has been established, it will be of further interest to increase the complexity of these systems. One means for doing so will be to require more

joining reactions to form the template molecules, for example, a system of the form: $A + B + C + D \rightarrow T$. Initial progress along these lines has been made by von Kiedrowski and colleagues, who devised a self-replicating system of the form: $A + B \rightarrow AB$; $AB + C \rightarrow T$; in which two substrate-joining reactions give rise to a template molecule that catalyzes both joining reactions [27]. As the number of substrate components increases and the size of these components decreases, the system will begin to approach the complexity of biological self-replicating systems.

A second potentially fruitful area of exploration would be the development of a heterogeneous population of self-replicating molecules that operate under a common set of reaction conditions. This might involve a system of the form: $A_1 + B_1 \rightarrow T_1$; $A_2 + B_2 \rightarrow T_2$; ...; $A_n + B_n \rightarrow T_n$; where each reaction operates independently. Alternatively, it might involve a system of the form: $\{A_1, A_2, \dots, A_n\} + \{B_1, B_2, \dots, B_n\} \rightarrow \{T_1, T_2, \dots, T_n\}$; where the product of one reaction may accelerate (or inhibit) another reaction, all reactions drawing upon a shared set of substrates to bring about the synthesis of the set of products (see also Update). The former approach would be applicable to the multiplex analysis of independent replicators, while the latter approach, by fostering competition for a finite set of building blocks, could lead to the emergence of more efficient replicators.

The development of a highly efficient chemical self-replicating system has been impeded by the large number of prototypes that must be synthesized and tested to optimize the critical variables in the system. Recent results have made it clear that even subtle changes can dramatically affect the efficiency of replication, so each variable must be examined carefully. A potentially fruitful approach to the design of self-replicating systems would allow several variables to be probed simultaneously, perhaps employing high-throughput screens or *in vitro* selection methods to sort through the many possibilities. Such an approach may lead to the isolation of self-replicating molecules with an appropriate balance of selective formation of the reactive complex, efficient catalysis, and rapid product dissociation, enabling sustained exponential growth.

Chemical self-replicating systems, in addition to providing a simplified model of biological self-replication, are likely to have applications in the areas of molecular computing and chemical signal amplification. Networks of cross-catalytic replicators can be regarded as performing logical operations based on the topology of allowed and disallowed molecular interactions. Such networks might be used to carry out computations by supplying them with an input set of templates and monitoring the output set of products. Autocatalytic replicators would provide a means for achieving 'gain' in such molecular

circuits, converting a small input signal to a larger output signal. Chemical self-replication also might be used to amplify a weak chemical signal that signifies the presence of a target compound. In that case, the self-replication process must be linked to a molecular recognition event, so that replication reports upon the presence of the target ligand. Such an approach may be fruitful for the construction of chemical and biological sensors.

Update

Through a combination of rational design and experimental analysis, Ghadiri and colleagues [28**] devised a complex network of self-replicating and cross-replicating peptides. The system had the form: $\{A_1, A_2, \dots, A_9\} + B \rightarrow \{T_1, T_2, \dots, T_9\}$, employing nine different **A** substrates and a common **B** substrate to form nine different template-products. Four of the templates behaved autocatalytically and 22 cross-catalytic interactions were identified. The topology of the network could be varied, depending on which templates were present at the outset. This is an example of the type of enhanced complexity that will be more common in future examples of chemical self-replicating systems.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Conn MM, Rebek J Jr: **The design of self-replicating molecules.** *Curr Opin Struct Biol* 1994, **4**:629-635.
 2. Bag BG, von Kiedrowski G: **Templates, autocatalysis and molecular replication.** *Pure Appl Chem* 1996, **68**:2145-2152.
 3. Robertson A, Sinclair AJ, Philp D: **Minimal self-replicating systems.** *Chem Soc Rev* 2000, **29**:141-152.
 4. Paul N, Joyce GF: **Self-replication.** *Curr Biol* 2003, **13**:R46.
 5. von Kiedrowski G: **A self-replicating hexadeoxynucleotide.** *Angew Chem* 1986, **25**:932-935.
 6. Zielinski WS, Orgel LE: **Autocatalytic synthesis of a tetranucleotide analogue.** *Nature* 1987, **327**:346-347.
 7. von Kiedrowski G, Wlotzka B, Helbing J, Mazten M, Jordan S: **Parabolic growth of a self-replicating hexadeoxynucleotide bearing a 3'-5'-phosphoamidate linkage.** *Angew Chem* 1991, **30**:423-426.
 8. Paul N, Joyce GF: **A self-replicating ligase ribozyme.** •• *Proc Natl Acad Sci USA* 2002, **99**:12733-12740.
This article describes a self-replicating system based on an RNA template that also functions as a ribozyme to catalyze its own replication. It is the first example of a self-replicating system with a reaction order of 1.0.
 9. Lee DH, Granja JR, Martinez JA, Severin K, Ghadiri MR: **A self-replicating peptide.** *Nature* 1996, **382**:525-528.
 10. Lee DH, Severin K, Yokobayashi Y, Ghadiri MR: **Emergence of symbiosis in peptide self-replication through a hypercyclic network.** *Nature* 1997, **390**:591-594.
 11. Yao S, Ghosh I, Zutshi R, Chmielewski J: **A pH-modulated, self-replicating peptide.** *J Am Chem Soc* 1997, **119**:10559-10560.
 12. Yao S, Ghosh I, Zutshi R, Chmielewski J: **Selective amplification by auto- and cross-catalysis in a replicating peptide system.** *Nature* 1998, **396**:447-450.
 13. Issac R, Chmielewski J: **Approaching exponential growth •• with a self-replicating peptide.** *J Am Chem Soc* 2002, **124**:6808-6809.
This communication describes a peptide-based replicator with shortened substrate-recognition domains. The template-product complex was somewhat destabilized, resulting in a reaction order of 0.91.
 14. Li X, Chmielewski J: **Peptide self-replication enhanced by a • proline link.** *J Am Chem Soc* 2003, **125**:11820-11821.
This communication describes a peptide-based self-replicating system in which a proline residue was introduced to cause a kink in the templating surface. This substitution destabilized the template-product complex, resulting in improved product release and a reaction order of 0.91.
 15. Tijvikua T, Ballester P, Rebek J Jr: **A self-replicating system.** *J Am Chem Soc* 1990, **112**:1249-1250.
 16. Terfort A, von Kiedrowski G: **Self-replication by condensation of 3-aminobenzamides and 2-formylphenoxyacetic acids.** *Angew Chem* 1992, **31**:654-656.
 17. Wintner EA, Conn MM, Rebek J Jr: **Self-replicating molecules: a second generation.** *J Am Chem Soc* 1994, **116**:8877-8884.
 18. von Kiedrowski G: **Minimal replicator theory I: parabolic versus exponential growth.** In *Bioorganic Chemistry*, vol 3. Edited by Dugas H. Berlin, Heidelberg: Springer-Verlag; 1993:115-146.
 19. Issac R, Ham Y-W, Chmielewski J: **The design of self-replicating helical peptides.** *Curr Opin Struct Biol* 2001, **11**:458-463.
 20. Dawson PE, Muir TW, Clark-Lewis I, Kent SBH: **Synthesis of proteins by native chemical ligation.** *Science* 1994, **266**:776-779.
 21. Rogers J, Joyce G: **The effect of cytidine on the structure and function of an RNA ligase ribozyme.** *RNA* 2001, **7**:395-404.
 22. Santoro SW, Joyce GF: **A general purpose RNA-cleaving DNA enzyme.** *Proc Natl Acad Sci USA* 1997, **94**:4262-4266.
 23. Levy M, Ellington AD: **Exponential growth by cross-catalytic •• cleavage of deoxyribozymogens.** *Proc Natl Acad Sci USA* 2003, **100**:6416-6421.
This article describes a deoxyribozyme-based, cross-catalytic amplification system in which efficient product release was achieved through cleavage, rather than a joining reaction. Optimal sequences of the substrate-recognition domains were isolated by *in vitro* selection.
 24. Sievers D, von Kiedrowski G: **Self-replication of complementary nucleotide-based oligomers.** *Nature* 1994, **369**:221-224.
 25. Chen J, Korner S, Craig SL, Rudkevich DM, Rebek J Jr: •• **Amplification by compartmentalization.** *Nature* 2002, **415**:385-386.
This paper describes a chemical amplification system in which release of a precursor reagent from a compartment led to the formation of two product molecules, each of which promoted the release of two additional precursor molecules. The rate of precursor release was found to increase exponentially over time.
 26. Chen J, Korner S, Craig SL, Lin S, Rudkevich DM, Rebek J Jr: • **Chemical amplification with encapsulated reagents.** *Proc Natl Acad Sci USA* 2002, **99**:2593-2596.
This article provides an in-depth kinetic analysis of the behavior of the chemical amplification system described in [25**], with emphasis on the autocatalytic nature of product uptake into the compartments.
 27. Achilles T, von Kiedrowski G: **A self-replicating system from three starting materials.** *Angew Chem* 1993, **32**:1198-1201.
 28. Ashkenasy G, Jagasia R, Yadav M, Ghadiri MR: **Design of a •• directed molecular network.** *Proc Natl Acad Sci USA* 2004, **101**:10872-10877.
This article describes the design and implementation of a network of nine α -helical peptides that direct each other's synthesis through various autocatalytic and cross-catalytic pathways. The network has a complex topology, including two-, three- and four-member cross-catalytic cycles.