Synthetic Networks

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The Road to Non-Enzymatic Molecular Networks

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minimal self-replication · self-organized networks · systems chemistry · template-assisted catalysis

This Minireview gives an overview of recent progress in the design and analysis of chemical systems that utilize template-directed autocatalytic and cross-catalytic processes as a means of wiring dynamically interacting molecules. Synthetic networks comprising two to nine replicating species are discussed. It is shown that for larger systems, more catalytic pathways must be manipulated to control the entire network topology and specific functionality of the individual species or subnetworks. Cellular biochemistry is an example of a natural functional molecular network; synthetic self-organized networks can provide additional models of complex systems.

1. Introduction

Many different systems are constructed from networks of interconnected elements. Even networks that differ significantly in size, scale, and functionality (for example, biochemical, ecological, engineered, and even economic networks) share many common organizational features.[1] Biochemical networks that regulate cell behavior can serve as intuitive models for the design of functional molecular networks. This behavior is a consequence of a complex web of interactions between numerous constituents, such as DNA, RNA, proteins, and small molecules. Cells use signaling pathways and regulatory mechanisms to coordinate multiple processes, thereby allowing them to respond and adapt to an ever-changing environment. The large number of components, the degree of interconnectivity, and the complex control of cellular networks are becoming evident in the integrated genomic and proteomic analyses that are currently being published.

During the past few years, both synthetic chemists and molecular biologist have been attempting to design, construct, and analyze simple dynamic molecular networks. Cell biochemistry is a natural example of a functional network; rationally designed self-organized synthetic networks might provide models for better understanding the behavior of complex systems. The intermolecular interactions and the dynamics of processes within synthetic networks can be controlled and manipulated more easily than within the robust cellular networks. The exploitation of non-natural species can provide new types of network behavior that may not be observed from natural species alone. Moreover, synthetic networks can be used to conceptually and practically perform several programmed tasks in parallel, for example, template-assisted catalysis or sensing.

This Minireview covers the use of template-directed intermolecular autocatalysis and cross catalysis as a means of wiring networks of dynamically interacting molecules. Synthetic networks that operate through replication of molecules (or part of the molecules) have received considerable attention with respect to possible scenarios for early molecular evolution and the origin of life. The design and analysis of minimal autocatalytic systems has been described in a number of reviews.[2] That research is thus reviewed only briefly in the Introduction to describe the functional chemical entities that can be used to produce networks and to highlight the kinetic parameters that are essential for understanding different aspects of network functionality. We have arranged this Minireview to emphasize progress in the field in terms of the complexity of non-enzymatic catalytic networks rather than in chronological order. We will show that the larger the system, the greater the number of catalytic pathways that can and should be controlled to show specific functionality in terms of the entire network topology, subnetwork relationships, and individual catalytic pathways.

1.1. Minimal Self-Replicating Systems

Self-replication of synthetic molecules can be considered as a simplified version of the complex multistep process that

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generates identical copies of macromolecules in cells. Several different examples of minimal self-replicating systems have been studied; these involve nucleic acids (DNA[3] and RNA[4]), peptides,[5] mixed protein–nucleic acid systems,[6] and small abiotic organic molecules.[2b,7] In each cycle of the self-replication process, the replicating molecule (T) binds to shorter molecules A and B, which are fragments of T, to form a ternary complex (A-B-T), positioning the reactive ends in close proximity to facilitate covalent bond formation (Figure 1). Coupling of A and B results in the formation of another copy of T. The template–product complex (T-T) then dissociates to provide two free copies of T, which can re-enter the next cycle of replication.

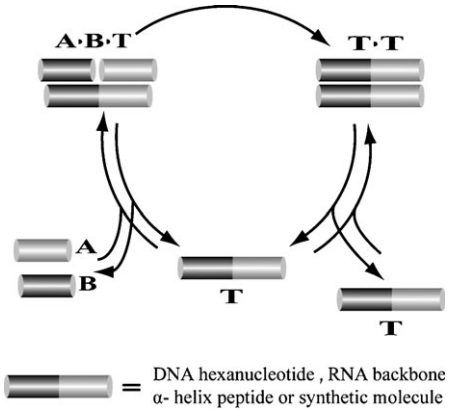

Figure 1. An autocatalytic cycle. Autocatalysis has been demonstrated using DNA, RNA, peptide, and abiotic molecules. The autocatalytic efficiency depends on the propensity of the template T and its fragments to form the complex A·B·T and on the dissociation rate of the catalyst–product dimer T·T. All four of these molecular families have been further utilized to devise binary or larger networks.

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Nathaniel Wagner received his Ph.D. in Physics from the Hebrew University of Jerusalem in 1992. Over the years he has worked for private industry in signal processing, computer modeling, and algorithm development. His current research interests at Ben Gurion University include complex systems, thermodynamics of living systems, and the modeling of kinetic profiles of molecular networks.

Gonen Ashkenasy received his Ph.D. in Organic Chemistry from the Weizmann Institute of Science in 2001. He then spent four years as a postdoctoral fellow at the Scripps Research Institute working with Prof. M. Reza Ghadiri on the design and functional analysis of synthetic networks. At the beginning of 2006, Ashkenasy joined the faculty of the Chemistry Department at Ben Gurion University. The research interests of his lab include molecular design of repeat proteins, design and synthesis of peptide catalysts, and theoretical and experimental aspects of synthetic networks.

Figure 2a shows the kinetic mechanism of the self-replication process. This minimal model is usually sufficient[8] to describe autocatalysis and self-replication. A full kinetic model has also been developed to explain a wide range of behaviors under a variety of conditions.[9] Figure 2b shows the kinetic profiles for two extreme cases: parabolic and exponential replication. Several parameters should be optimized to prepare a system that exhibits efficient autocatalytic behavior. First, template T should bind tightly and selectively to substrates A and B to produce a significant population of intermediate A-B-T. Second, the ligation step that transforms the A-B-T complex to the T-T complex should be enhanced by the template relative to the template-free reaction. Third, the release of the newly formed product should occur readily, providing accessible catalyst molecules for reactions in subsequent cycles.

Replication efficiency is usually characterized by two kinetic parameters.[2h,i,8] The first is ε, which reflects the ratio between template-assisted and the template-free ligation reactions; the second is the order parameter p, which relates the rate of product formation to the (total) product concentration raised to the power p. The parameter p is thus derived experimentally from a logarithmic graph of initial reaction rates versus initial concentrations of seeded template (Figure 2c).

The maximal value of $p = 1$ corresponds to efficient T-T dissociation, thus allowing autocatalytic, exponential amplification. On the other hand, $p = 0.5$ corresponds to rate-limiting slow dissociation of the T-T complex, that is, product inhibition resulting in parabolic growth.[2i] In his minimal replicator theory paper, von Kiedrowski discussed the different cases of weak and strong exponential growth, which depend on system conditions and rate constants.[8] Most of the systems studied were found to replicate in a parabolic manner, but some specifically designed[10] or selected[11] systems resulted in more efficient autocatalysts that replicated with high amplification and with p almost approaching 1.

The catalytic principles of all four molecular families represented in Figure 1 have been utilized to devise binary or larger systems that can replicate not just autocatalytically but also by cross-catalytic processes in which the template enhances the formation of different molecules, usually its own mutants. The catalytic efficiency and type of replication are central to the design and control over competing pathways within the networks, as discussed in detail in the following sections.

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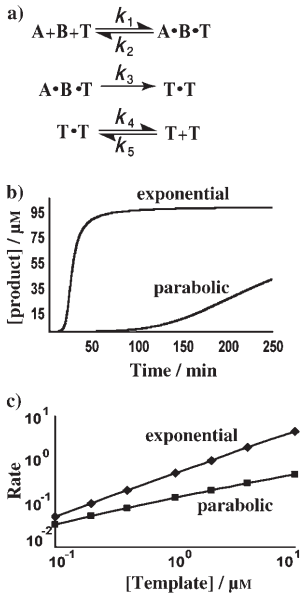

Figure 2. a) A simple reaction mechanism describing an autocatalytic process.[8] b) Kinetic profiles of parabolic and exponential growth of self-replicating molecules. The two curves were obtained by simulation using realistic parameters from peptide replication experiments, including slow template-free formation of T and 0.1 mol% seeded catalyst. The desired exponential growth is usually observed when T·T dissociation is fast. Thus, a thousand-fold difference in the dissociation parameter k_4 was used to simulate the two distinct processes. c) A log–log plot of initial rates of product formation as a function of different concentrations of seeded T, as simulated using the parameters described in (b). As in the analysis of the experimental results, the slopes in this plot reveal p values close to 0.5 and 1 for parabolic and exponential growth, respectively.

2. Simple Networks Formed by Implementation of Autocatalysis and Cross Catalysis

A mixture of molecules can be regarded in a broad sense as a "network" if all the molecular components participate in some kind of interaction with other molecules—either physical (covalent or noncovalent bonding) or functional (contribution to certain catalytic processes). The simplest network would thus consist of two molecules that interact in productive or nonproductive (competitive) ways. The template-assisted ligation (coupling) reactions that direct self-replication processes can serve as the functional elements that connect two members of a catalytic network. In such a process, the template T does not necessarily catalyze its own formation, but rather the formation of another molecule (T′), which in turn can operate as a template for other reactions in the network. Efficient and selective cross-catalytic processes with peptides[12] have been demonstrated in isolated systems, that is, not within a catalytic network. Such studies were performed with DNA[13] as part of a more elaborate study that characterized a small network (see Section 3). As in most autocatalytic systems, efficient cross catalysis is often accompanied by high substrate inhibition owing to the structural matching required to form the new product.

The implementation of autocatalysis and cross catalysis in binary systems can take different forms, depending on the specific properties of the two molecules (Figure 3). To facilitate coherent descriptions of various binary and larger networks, we will use schematic descriptions similar to presentations used in system biology papers, in which networks are represented by graphs of "collected nodes" and "directed edges". In terms of this convention, the nodes represent template or product molecules, and the edges represent catalytic processes proceeding from the templates to their products. Figure 3 shows schematically five different possible intermolecular relationships in the binary system. The "complete" situation, in which each of the molecules is an autocatalyst and can also catalyze the formation of the other, is described in detail, with an explanation of the equivalency of the two representations of the network (Figure 3a). The

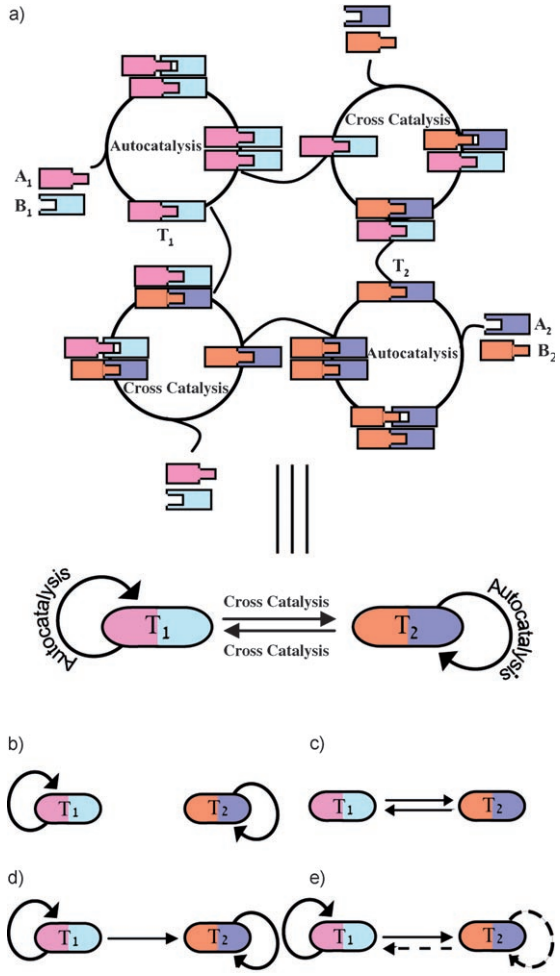

Figure 3. Graph description of binary molecular networks. Nodes represent the molecular species, while the edges represent catalytic processes in which the arrow-tail nodes enhance the formation of the arrow-head nodes. Circular arrows are shown for autocatalytic processes and dashed arrows for weak processes. The "complete" situation, in which each molecule is an autocatalyst and can catalyze the formation of the other molecule is shown in detail in (a), together with its graph presentation analogy. All binary relations shown in (a–e) are discussed in the text.

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two extreme cases, in which the templates function either solely as autocatalysts or solely through cross catalysis, are shown in Figure 3b,c. The other two examples show unsymmetric networks in which the cross-catalytic pathway is available only in one direction or is stronger in one direction (Figure 3d,e). Such relationships in binary networks or subnetworks of larger systems have been experimentally realized and mathematically simulated.

2.1. Reciprocal Catalysis in Binary Systems

Binary molecular systems that replicate by reciprocal catalysis include two template/product molecules, T_1 and T_2, that are formed from two sets of fragments (A_1, B_1 and A_2, B_2, respectively, Figure 3a). T_1 and T_2 are designed in such a way that they can efficiently dimerize with each other, while self-dimerization is minimized through structural or electronic repulsion. As a result, each template can bind selectively to the fragments that lead to the formation of the other template and enhance the rate of their condensation [Figure 3c and Eqs. (1) and (2)].

$$A_1 + B_1 + T_2 \rightarrow T_1 + T_2 \quad (1)$$

$$A_2 + B_2 + T_1 \rightarrow T_2 + T_1 \quad (2)$$

The reciprocal cross catalysis was discussed in early publications as a second level of organization in autocatalysis and was considered as an intuitive model to mimic double-stranded DNA replication.[14] The experimental analysis of the reciprocal behavior of the two molecules is simple when studied in isolation. In such studies it should be shown that in the presence of T_2 the formation of T_1 (from A_1 and B_1) is enhanced relative to either the background ligation or to the situation when T_1 itself is added, and vice versa for the enhancement of T_2.

Because in some systems the same chemical reaction leads to the formation of either T_1 or T_2, "promiscuous" reactions through cross-coupling of $A_1 + B_2$ and $A_2 + B_1$ to form T_{12} and T_{21}, respectively, also occur (for example in the RNA-based system described in reference [15]). Even if such processes take place only through background uncatalyzed reactions, the result is the formation of a four-member network (Section 3.1), for which the kinetic analysis is more complicated.

An RNA ligase ribozyme system was designed by Kim and Joyce for selective cross-catalytic RNA-assisted ligation.[15] This system utilizes the catalytic core of the R3C ligase, which catalyzes an attack of the 3′-hydroxy group of one RNA substrate on the 5′-phosphodiester and which has self-replication capabilities.[4] By breaking the symmetry of the original self-replicating ligase ribozymes, the authors prepared a mixture containing the four corresponding oligonucleotides and monitored how each of the ribozymes enhanced the formation of the other. To preserve the catalytic activity of the ribozymes, the symmetry breaking was introduced by mutating positions close to the 5′ and 3′ ends of the ribozymes while keeping the catalytic core untouched.

A reciprocal template effect was demonstrated by Rebek and co-workers utilizing a system made up of aromatic abiotic products and bisadenine derivatives as templates.[16] Recently, a system of two mutually complementary templates, consisting of organic abiotic molecules that replicate using Diels–Alder reactions, was reported by Kassianidis and Philp (Figure 4).[17] Similar to the original self-replicating molecule

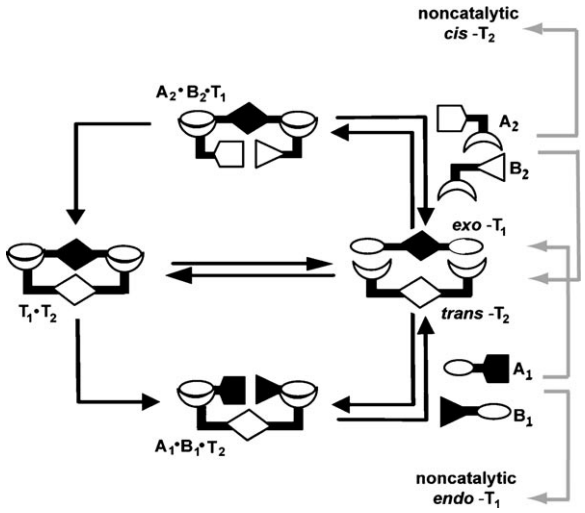

Figure 4. Reciprocal replication by two complementary abiotic templates.[17] The system is designed to provide significant complementarity of the *exo*-T_1 isomer to the transition state leading to the *trans*-T_2 isomer and vice versa. Thus, the amplification of the formation of these specific diastereomers, at the expense of their corresponding diastereomers *endo*-T_1 and *cis*-T_2, is evident within the network and is utilized to monitor information transfer. The *endo*-T_1 and *cis*-T_2 isomers, which are formed in the background, are catalytically inactive (or less active) but nevertheless contribute to the overall observed behavior.

in the simpler system,[7c] each of the two templates could be formed as two diastereomers, *endo*- and *exo*-isomers of T_1 and *cis*- and *trans*-isomers for T_2. This arrangement facilitated the probing of information transfer between the templates by measuring the differences in diastereoselectivity between the template-free bimolecular and template-directed reactions. On the basis of electronic structure calculations, the authors reasoned—and then showed experimentally—that one template shows significant complementarity to the transition state leading to only one of its partner's diastereomers (Figure 4).

A totally different approach was taken by Levy and Ellington in their study of mutual replication by two DNA molecules with RNA-cleavage activity.[11] In their system, each molecule could exist in an active linear form (L_A, L_B) or an inactive circular form (C_A, C_B; Figure 5). Each linear DNA molecule was used to cleave a specific site on the circular form of the other molecule, generating a new active species that could then serve to form an additional catalytically active species of the former. Following the cleavage step, product release took place readily. Seeding the system with the linear catalysts resulted in significant amplification, and the system was calculated to proceed with a reaction order of 1.0 and catalytic efficiency of more than 10^9.

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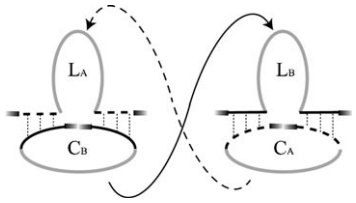

Figure 5. Reciprocal replication of DNA molecules through a cleavage cycle.[11] Cleavage of inactive C_B by active L_A produces active L_B, which in turn cleaves inactive C_A to reproduce active L_A.

2.2. Simultaneous Implementation of Autocatalysis and Cross Catalysis in Binary Systems

A small network of two molecules, each replicating by both autocatalytic and reciprocal cross-catalytic pathways (Figure 3a), was constructed from two coiled-coil peptides.[18] These peptides were based on two originally self-replicating molecules differing in the hydrophobic patches of their recognition interfaces. Owing to the fact that a coiled-coil assembly can accommodate different residues within its hydrophobic core,[19] mixed oligomers could be formed, and consequently the formation of each peptide was also catalyzed in the presence of the other. Ghadiri and co-workers suggested that two such molecules can arrange themselves into a subnetwork of larger systems and, owing to the mutual replication behavior, adapt better to compete for available resources. Such behavior could be tested only a few years later with the analysis of larger molecular networks (Section 3).

2.3. Asymmetric Relationships between Templates in Binary Systems

It is reasonable to envision a situation in which two molecules in a network exhibit different autocatalytic or cross-catalytic properties. Indeed, such differences are key elements in the design of systems that perform more complex functions, owing to their much larger conformational and topological spaces. The examples in Figure 3d,e describe scenarios in which one molecule acts as a catalyst for the formation of another molecule in the network, while the reciprocal process does not take place or is less efficient. Within the context of the mutually replicating system described in the previous section, it was shown that the two replicating molecules differ in autocatalytic properties as well as in their ability to catalyze each other's formation.[18] These differences were attributed to the fact that the catalytic efficiency of the peptide-based template-assisted ligation process correlates directly with the stability of the coiled coils, either of the intermediate complex (T-A-B) or of the product–catalyst (T-T′) complex. Later on it was suggested that asymmetry may have been introduced into this and similar systems, owing to the fact that homodimer complexes, rather than monomeric helices, serve as the templates.[20,21] The unsymmetric relationships between nodes in non-enzymatic networks were also observed and studied within the context of subnetworks of larger systems (see Figures 8, 9, and 11 for graphs of larger networks).

2.4. Ternary Systems

The topology of three-component networks can represent various different functional motifs. In recent years, such motifs have been the subject of intensive study in the context of regulatory networks.[1f,22] Ternary networks present a much wider range of intermolecular interactions than binary systems. The main difference is that the functionality of a given molecule within a ternary network can be enhanced or inhibited by the two other molecules, which can act independently or in concert.

An example of a ternary peptide-based replicating network was prepared from an original replicator and two of its mutants, each containing a single Leu→Ala mutation in the hydrophobic core.[23] Since the Ala side chain is smaller than that of Leu, the coiled-coil assemblies containing the Ala mutants are less close-packed and less stable. Accordingly, the template-assisted ligation processes with Ala mutants as templates or substrates are expected to be less efficient. In a number of experiments the system was seeded with each of the three molecules; it was shown that the original sequence, the all-Leu mutant, can self-replicate but does not catalyze the formation of its mutants. The Ala-mutants, on the other hand, do not replicate, but each can enhance the formation of the original sequence. Intriguingly, the intermolecular interactions in such a simple chemical system (Figure 6a) simulated a scenario similar to an evolutionary error-correction process that takes place following the augmentation of mutations in a productive species in the cell.

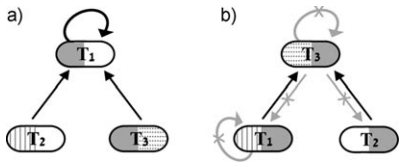

Figure 6. Organizational motifs in tertiary peptide-based networks. a) Tertiary system studied as a model for dynamic error-correction processes. The network is made up of an efficient autocatalyst (T_1) and two of its mutants, which are produced by Leu→Ala mutations (T_2 and T_3). The two mutants are inefficient autocatalysts, but they can serve as catalysts for formation of their "mother peptide".[23] b) Tertiary network directed to produce one peptide (T_3) by an OR logic operation that exploits the two other network members. The design of the system makes use of unsymmetric cross-catalytic processes in order to "shut off" competing pathways in the network (non-operative pathways in gray).[24]

Another ternary network with somewhat similar topology was published a few years later during the search for logic operations within such networks.[24] The ternary system shown in Figure 6b facilitates the production of one molecule (T_3) through an OR operational motif from two other molecules. The design of the system makes use of unsymmetric template-assisted ligation. It was found experimentally that this motif is functional only in the chemical mixture of the entire network and not when individual components are employed to characterize pathways in isolation. This "network behavior" that shuts off several catalytic pathways (e.g., the autocata-

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lytic formation of T_3 that was observed when studied in isolation) was attributed to the dominance of some pathways over other "latent" pathways (shown as non-operational in Figure 6b).

2.5. Analytical and Computational Studies of Simple Non-Enzymatic Networks

Both minimal self-replicating systems and autocatalytic and cross-catalytic networks employing monomeric and dimeric catalysts have been solved mathematically and computed numerically. An empirical rate equation was used early on to describe the time–concentration dependence of minimal self-replicators.[8] The differential equation was solved both analytically (with certain approximations) and numerically, giving both parabolic and exponential growth curves as well as surface contours for the reaction order p as a function of the system's equilibrium constants. The experimental nucleotide-[3a] and peptide-based[18] systems were simulated by modeling, and the corresponding differential equations were solved using a Taylor series approximation.[25] Practical kinetic rate constants were obtained by calibration with the experimental data. For organic molecules, Reinhoudt et al.[9] performed kinetic modeling and numerical calculation based on experimental values of the equilibrium constants. The close agreement between predictions and their experiments confirmed a comprehensive kinetic model that succeeded in explaining results obtained under different experimental conditions.[26]

Detailed kinetic expressions for single-template and multiple-template autocatalysis and cross catalysis under various steady-state assumptions and approximations were analytically solved by Wills et al., who explored the boundaries between coexistence and competition in the multiple-template case.[27a] A continuation of this approach was used to study higher-order replication reactions (i.e., dimers as catalysts), leading to the observation of fixed points, bifurcations, and "hypercycles".[27b]

Experimental results for autocatalytic and cross-catalytic replication in networks of nucleotides were computed numerically by kinetic modeling and parameter fitting using the program SimFit.[13] The experimental chiroselective peptide network (Section 3.1)[28] was modeled, and its differential equations were numerically solved to highlight the heterochiral[29a] and homochiral[29b] cases.[29c] Recently, Peacock-López et al. modeled the kinetics of autocatalytic and cross-catalytic self-replication systems with a set of dimensionless differential equations.[30] By solving the equations numerically, they obtained temporal and spatial patterns leading to bifurcations and oscillations.

3. Networks with Multiple Catalytic Pathways

The expansion of molecular systems beyond two or three components poses a challenge for chemists trying to direct such networks to function in predetermined ways. The main difficulty is how to control large numbers of processes that take place in parallel and are operated by molecules that participate in more than one equilibrium. On the other hand, the study of such systems enables chemists to demonstrate and analyze processes that are usually seen only in much more complex systems, such as cells. In the following sections, we describe synthetic networks made of four or more molecules, which exhibit "network behavior", a term that relates to processes that affect the entire network topology or those that control individual pathways through multiple inputs, which can be obtained only within such mixtures.

Figure 7 shows a general scheme of all the possible catalytic processes within a postulated network. Generally speaking, each molecule in the network can act in four

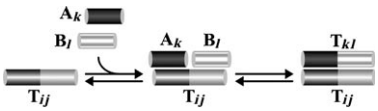

Figure 7. General mechanism that describes the operational catalytic pathways within networks. When the indices kl are equal to ij, the equation describes an autocatalytic process; otherwise it describes all other possible cross-catalytic processes. Altogether this formulation describes a maximal number of n^2 catalytic pathways within a network composed of n molecular species.

different ways: autocatalytic, cross catalytic, both autocatalytic and cross catalytic, or noncatalytic. It should be noted that the differences in the intensity of action for each process play an important role and that molecules that do not participate in catalytic pathways (as neither template nor product) should also be considered part of the network if they compete with other molecules for starting materials.

An example of a network that is wired by six catalytic pathways was studied by Achilles and von Kiedrowski, who analyzed a self-replicating system that starts from three building blocks (A, B, and C in Figure 8).[31] A single full-length hexanucleotide autocatalytic molecule, which is a condensation product of all three fragments (ABC), was formed within the network. Four additional smaller species made of only two fragments were also formed, and three of them were found to be involved in autocatalytic or cross-catalytic activity. Seeding experiments of the system with pre-prepared analogues of each product revealed all the existing catalytic pathways and provided evidence for the minimal length of DNA molecules required to show template effects.

3.1. Quaternary Systems

A number of synthetic networks have been produced from four template/product molecules that are formed from two sets of molecules, as shown in Equation (3).

$$A_1 + A_2 + B_1 + B_2 \rightarrow T_{11} + T_{12} + T_{21} + T_{22} \quad (3)$$

The starting materials are prepared such that A molecules (e.g., electrophiles) can react only with B molecules (e.g., nucleophiles). Up to 16 different catalytic pathways can be obtained within such networks when it is taken into account

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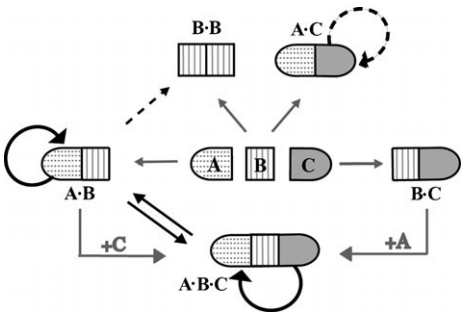

Figure 8. DNA-based network formed during the self-replication process originating from three building blocks A, B, and C (tri-, di- and mononucleotides).[31] The synthetic pathways of their condensations are shown with gray arrows, while template-assisted reactions that enhance the formation of one condensation product by another are depicted in black, with dashed arrows for weaker catalytic pathways.

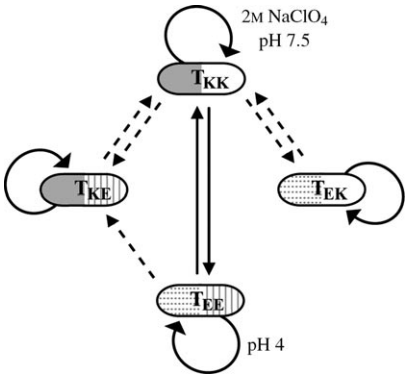

Figure 9. Quaternary peptide-based molecular network that selectively amplifies the formation of different molecules under different conditions.[21] T_{KE} and T_{EK} represent full-length peptides with both Glu and Lys residues in their recognition interface, and T_{KK} and T_{EE} represent peptides with only Lys and only Glu, respectively. The best autocatalytic or cross-catalytic efficiency was observed under conditions that minimize repulsion between the coiled-coil helices of the relevant pathway (see text).

that a monomeric T molecule can serve as a template for its own formation or as a catalyst for the formation of another molecule.

Sievers and von Kiedrowski studied a quaternary system that was based on their hexadeoxynucleotide analogues. They demonstrated that cross-catalytic replication can be observed in template-directed reaction systems if the reciprocal template effects are similar in efficiency and if the reaction system is not dominated by autocatalytic syntheses of self-complementary products, which occur as parallel reactions.[13,32] In this system, the nucleotide sequence CCG may be designated as 1 and sequence CGG as 2. The sequences 1 and 2 can react to form four possible template/product molecules: CCGCCG (T_{11}), CCGCGG (T_{12}), CGGCCG (T_{21}), and CGGCGG (T_{22}). In the absence of any template, all four hexanucleotides were formed with similar rates. The coupling reactions were monitored in the presence of one of the hexadeoxynucleotide templates, and it was shown that each hexamer template stimulated the formation of the one and only reaction product whose sequence was complementary to the template's sequence. Interestingly, the effect of a single non-self-complementary template, T_{11} or T_{22}, was found to be more pronounced than the effect of a single self-complementary template.

The ability of a system to selectively amplify the production of one or more molecules is an important and challenging task. Chmielewski and co-workers described a four-component peptide-based network capable of autocatalysis and cross catalysis that allows for selective amplification of product formation by changing the reaction conditions (Figure 9).[21] Their design was based on coiled-coil fragments that contain either polylysine or polyglutamate residues in the e and g heptad positions that flank the hydrophobic core, similar to the original design of the "velcro" assembly.[33] The four product peptides were studied for their catalytic ability under conditions that minimize repulsion between the coiled-coil helices. The mixed Glu–Lys peptides (T_{KE} and T_{EK}, Figure 9) can form autocatalytically under physiological conditions, and the poly-Lys (T_{KK}) and poly-Glu (T_{EE}) peptides can promote each other's formation. The autocatalyses of T_{KK} and T_{EE} were accessed when the system was studied at high salt concentrations and at low pH value, respectively. A number of experiments showed that seeding the mixture with an individual template peptide did not promote the production of a single product, but rather several unexpected template-assisted processes were enhanced (dashed arrows in Figure 9).

An attractive process in replication systems is the chiroselective replication of biopolymers, which might explain homochirality in nature. In this context, Ghadiri and co-workers studied a system that is capable of efficiently amplifying homochirality through a chiroselective autocatalytic cycle (Figure 10).[28] An electrophile and a nucleophile were made of all L amino acids, and a second electrophile and nucleophile couple contained all D amino acids. When a "racemic" starting mixture made up of all four fragments was studied, the two homochiral templates, T_{LL} and T_{DD}, replicated much faster than the heterochiral products, T_{LD} and T_{DL}. The heterochiral products were produced in a template-independent fashion, largely through uncatalyzed bimolecular background reactions. The authors also tested the limits of chiroselective amplification by probing the effects of single amino acid stereochemical mutations within otherwise homochiral peptide fragments. They were able to show that chiroselectivity in peptide self-replication is remarkably robust, not favoring reactants and products that differ stereochemically even by one amino acid out of 15 or 32 residues.

3.2. Larger Networks

Relatively large peptide-based replicating networks were also investigated by Ghadiri and co-workers.[20] An array of 81 sequences of coiled-coil peptides was studied in silico to search for efficient template-assisted reactions. The calculations predicted the formation of a self-organized network made of 25 molecules and an interaction graph with a

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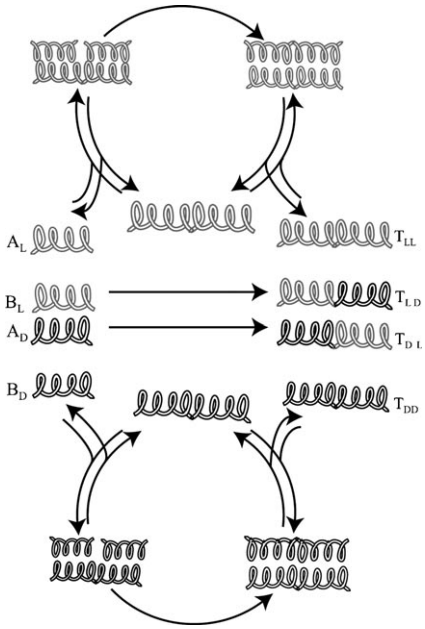

Figure 10. Chiroselective amplification of product formation in a quaternary network of two homochiral (T_{LL} and T_{DD}) and two heterochiral (T_{LD} and T_{DL}) peptides.[28]

topology similar to graphs of much more complex systems (Figure 11 a). The validity of the design principles was experimentally analyzed by testing nine nodes, comprising a main segment of the graph, for their capacity to establish the predicted network connectivity (Figure 11 b). The resulting self-organized chemical network was shown to display 25 directed edges, including three autocatalytic processes, in good agreement with the graph analysis estimations. The same network was studied further under a number of different conditions, and it was observed that once the pH value was lowered to below pH 5, or if metal salts were introduced—two processes that inevitably affect the free energy of ion pairing interactions and coiled-coil stability[34]—the network rewiring was substantial. The network formed alternate connectivities that gave rise to new central nodes and functional subnetworks.[35]

Selected segments of this simple network were studied for their ability to carry out basic Boolean logic operations (see an example of OR functionality in Figure 6 b).[24] It was established experimentally that the rates of product formation in the network differ significantly from the rates in isolated reactions, since the prevalence of certain nodes depends on the competition between and/or integration of inputs from other nodes. Consequently, a segment of the network, whose graph structure is composed of five nodes and fifteen directed edges, was used to express OR, NOR, and NOTIF logic. Such behavior would be conceptually similar to the way in which cells process multiple extracellular input signals rapidly and simultaneously through complex networks of biomolecular interactions and chemical transformations.

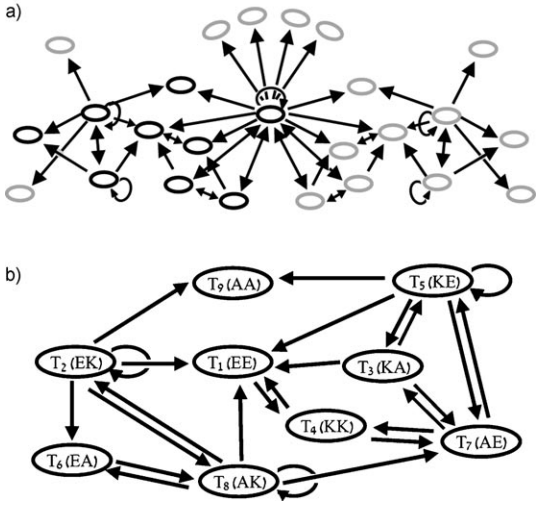

Figure 11. a) Calculated graph architecture illustrating formation of a self-organized peptide network composed of 25 nodes joined by 53 vector edges. Nodes highlighted in gray were evaluated experimentally for their ability to form that portion of the graph. b) The experimentally derived network architecture.[20] Two sets of experiments were needed to reveal both the strong and the latent catalytic pathways; the results are in good correlation with the predicted graph.

4. Summary and Outlook

We have described recent progress in transforming minimal self-replicating systems into small networks of dynamically interacting molecules. It has been suggested that such transformations occurred during the early stages of molecular evolution and may have led to the origin of living systems. Different terms, such as the emerging "RNA world", "hypercycles", or "systems chemistry", have been suggested to describe this phenomenon.[7b,36] Nevertheless, the results of both theoretical and experimental work suggest that several major challenges still remain to make the synthetic systems more efficient, selective, and diverse.

Increasing the efficiency of template-directed processes will result in better differentiation between slow and fast processes within networks and, accordingly, should improve selectivity of product formation. The expansion of the current networks to occupy larger topological spaces and to display new functionality could be achieved with the current building blocks if these were modified to operate through high-order reactions. For example, both DNA and peptides can be modified such that dimers or trimers are used as catalysts instead of monomeric species. The network topology may be further expanded by exploiting more than one type of molecule (the simplest example would be a network made of both DNA and protein molecules) or molecules with dual characteristics (e.g., DNA–protein conjugates). Such an expansion would allow the separate control of information transfer and catalytic ability. It should be noted, however, that the increase in network size should be accompanied by the development of new analytical tools for characterizing networks, especially if we seek to follow each and every pathway within the network, as currently realized by studying small systems. Finally, we suggest that the most interesting development would be the design of networks that actually display

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complex functionality, such as harmonic oscillations in product formation.

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