only component of this story, so it will be of interest to understand how the many additional phenazine-responsive, SoxR-independent genes are involved. Also of interest is the mechanism of SoxR activation under oxygen-limiting conditions[7]. Because oxygen is required for phenazines (and many other compounds) to generate superoxide[2], it has been suggested that something other than oxidation of the SoxR [2Fe-2S] clusters might be involved. In this context, it is interesting to note that Privalle *et al.* described anaerobic activation of *E. coli* SoxR by exposure to a metal chelator[8].

It is also intriguing that Enterobacteria evolved a more complex SoxR regulatory system dependent on SoxS as an intermediate. This complexity may reflect the transcriptional activation mechanism of SoxR, which binds the same stretch of promoter DNA as RNA polymerase and activates transcription by structurally remodeling the promoter[6]. It might be problematic to expand such a mechanism to additional genes without interfering with other modes of promoter regulation. In contrast, SoxS activates transcription by a more conventional mechanism of RNA polymerase recruitment[6]. This control can be readily overlaid with other regulatory mechanisms. It remains unknown whether there are other SoxR-regulated transcription factors that amplify the repertoire of genes downstream of SoxR in *soxS*-less bacteria.

Given that the formation of biofilms contributes to the pathology of *P. aeruginosa* in cystic fibrosis and in burn infections[3], modulating the behavior of bacterial populations is important in human disease. Thus, the community organizer role of phenazines and other actively generated compounds merits further attention. Because bacteria in nature are almost never a monoculture of one species, but instead co-exist with other species, the possible cross-talk between species via the metabolic signaling of the type discussed here might be of potential importance in pathology or in the maintenance of bacteria that normally populate humans.

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An autocatalytic network for ribozyme self-construction

Burckhard Seelig

The emergence of a primordial RNA world would have required the formation of RNA polymers of sufficient length to possess catalytic activities, which are difficult to obtain by spontaneous polymerization. An analysis of an autocatalytic assembly pathway that can self-construct a functioning ribozyme from smaller oligonucleotide building blocks describes a potential route for RNA extension.

The RNA world hypothesis postulates that RNA based life predates our current DNA/RNA/protein dominated world. In such a world, the RNA would have functioned as the carrier of genetic information and at the same time catalyzed all chemical reactions needed for a sustainable metabolism[1]. In today's organisms, we can still find a number of ribozymes that catalyze essential tasks in the cell, such as protein synthesis by the ribosome[2] and self-splicing of RNA[3]. In addition to naturally occurring ribozymes, researchers have generated several artificial ribozymes by screening vast mixtures of synthetic random RNA[4]. An unresolved question within the RNA world hypothesis relates to the formation of the first catalytically active RNA sequences; in other words, how did the first catalysts originate? Hayden *et al.* now provide some quantitative insight into this question in their systems chemistry study of the assembly of a deconstructed intron[5].

A feasible source of the first natural RNA molecules in the RNA world is the nonenzymatic condensation of activated nucleotides into oligomers (**Fig. 1a**). Ferris *et al.* have shown that RNA fragments of up to about 50 nucleotides can be produced on clay surfaces[6]. However, catalytically active RNAs are generally thought to be longer than 50 nucleotides, with the exception of those catalyzing simple, entropically favorable reactions. One mechanism for generating longer RNA molecules is the recombination of shorter fragments in an energy-neutral phosphodiester exchange reaction. For example, Doudna *et al.* have described a multisubunit ribozyme that acts as both catalyst and template for the ligation of RNA fragments complementary to one of its subunits[7]. More recently, Hayden *et al.* have added a new dimension to this construction principle[8]. They have developed a system that is not only capable of full self-replication of a ribozyme from RNA fragments but also introduces an autocatalytic feedback for the accelerated propagation of the ribozyme. These results constitute an important innovation. During the origin of life, autocatalysis is considered a key element in the transition from the prebiotic 'chemical' phase to one of self-organization and self-replication of 'individuals'[9].

Hayden and colleagues have focused on the self-splicing group I intron of the *Azoarcus* bacterium, a ribozyme that catalyses its own excision from a tRNA precursor. They have artificially broken down the ribozyme into four shorter RNA oligonucleotides and shown that those fragments can self-assemble into a catalytically active complex through base pairing and tertiary interactions (**Fig. 1b**). This multisubunit ribozyme complex can catalyze the recombination of more fragments into an uninterrupted, full-length ribozyme. The contiguous ribozyme in turn catalyzes the synthesis of further copies of itself and therefore provides an autocatalytic feedback. In their latest paper, Hayden *et al.* dissect the complex network of reactions that start from the four RNA fragments, pass through several intermediates and eventually produce the contiguous

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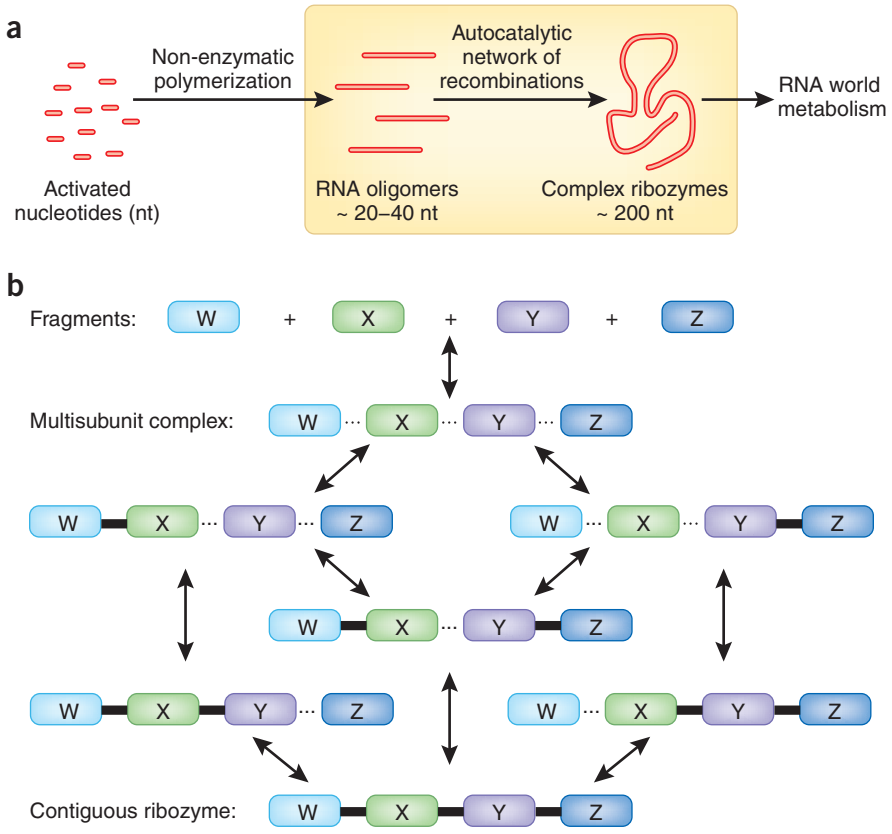

Figure 1 Model for the emergence of complex ribozymes in an RNA world. (**a**) Possible path of the formation of complex ribozymes from single nucleotides. (**b**) Reaction network of four RNA fragments forming a contiguous ribozyme through several intermediates. Double-sided arrows indicate reversible reactions. Dotted lines represent noncovalent interactions and solid lines represent covalent bonds.

ribozyme[5]. The authors synthesized several of the intermediates and measured initial reaction rates of those subsystems. Notably, some of the recombination intermediates also show autocatalytic behavior but to a lesser extent than the full-length ribozyme. Hayden *et al.*[5] developed a kinetic model with some necessary simplifying assumptions and evaluated the total autocatalysis of the system in the context of the entire network. The experimental data were in qualitative agreement with the model. They showed that the autocatalytic feedback by the contiguous ribozyme is the dominant component of the reaction network and produces about two-thirds of the full-length ribozyme in a given time. The remaining third of the product formation is catalyzed by the noncovalent multisubunit complex. The latter, nonautocatalytic component is essential for the initial formation of the autocatalytic species.

This example of an autocatalytic self-construction of a ribozyme[5] demonstrates a possible route from readily available, shorter, prebiotic RNA fragments to RNA molecules of sufficient length for complex catalytic activity (**Fig. 1a**). This scheme is a very interesting model system, but the likelihood of those four particular ribozyme fragments appearing at the same time and location in the origin-of-life context is extremely low. Therefore, it would be interesting to investigate whether a similar ribozyme could be found that retains the recombination ability but has very few conserved sequence requirements. Such a ribozyme with a high degree of sequence variability would be more likely to occur by chance through random nucleotide polymerization. The reaction model of the autocatalytic network by Hayden *et al.*[5] is a simplified system. However, to model, fully understand and describe networks of chemical reactions is a considerable task, even in highly simplified systems. Work on less complex synthetic models such as this one will help to identify underlying guiding principles that can then be applied to more complex systems, which are common in nature and more challenging to understand. In this way, these results advance the nascent field of systems chemistry, the area of research that investigates complex mixtures of molecules and the reaction networks that connect them[10]. Systems chemistry mirrors systems biology and systems chemical biology, which have similar approaches but focus on large networks of naturally occurring biomolecules of the living cell. The different fields have a significant overlap in their approach and methods, and they will complement each other as they develop.

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