photo-activates A and inactivates B. This creates two regions, one on the inside of the input pattern, which contains the activated A and inactivated B, the other region (outside of the input pattern) still contains photo-caged A and active B.

The activated A species is designed to catalyse a series of reactions that use active B and C as reactants, and produce a fluorescently labelled output signal as a final product. Initially, this reaction cannot occur because active A and active B are separated in space (Fig. 1c); however, as A and B diffuse, regions are formed containing both activated A and B (Fig. 1d). Because A is much smaller than B, it diffuses substantially faster. Over time, A crosses the pattern boundary where it can then catalyse the production of the output signal (Fig. 1d). This transformation process reliably produces an output pattern just to the outside of the original pattern boundary, and nowhere else (Fig. 1e). The researchers measured the width of this patterned edge as ~0.5 mm, and estimate that edges as narrow as ~0.1 mm should be achievable by tuning reaction and diffusion rates. However, these features are transient, and as the species in the network continue to diffuse and mix, the pattern blurs and then fades away.

Additional pattern transformations can be designed by making straightforward changes to the network of chemical reactions responsible for creating them. For instance, Ellington, Chen and co-workers reversed the input pattern so that the rapidly diffusing species A is on the outside of the input pattern and B is on the inside. The pattern produced by this modified network forms on the inside of the original pattern boundary, rather than on the outside. Likewise, additional reactions can be inserted into the network to add more features. Two edge-detection networks with the same design but different molecular components can produce a final pattern with one output species on the inside edge of the input pattern, and another species on the outside edge. By labelling alternative output signals with different coloured fluorescent tags, and using complementary networks that report the supplied input pattern, the researchers demonstrate the formation of a total of sixteen different patterns with the colour in each region pre-programmed by the reaction networks — analogous to a molecular 'colour by number'.

Engineers have previously formed chemical pattern-transformation processes by engineering cells that release and respond to intracellular signals[7]. The cell-free approach to pattern transformation introduced here has several important advantages. As Ellington and Chen point out, an *in vitro* chemical reaction network is much simpler than a network of interacting genes and proteins. When complexity is measured by the number of bases in the DNA components of the reaction, the *in vitro* network is more than 100-fold simpler than the analogous genetic network cells use to produce the same transformation. Furthermore, chemical networks based on synthetic DNA can be extended and combined to suit a variety of patterning applications through the design of their nucleotide sequences. In contrast, there are a limited number of intercellular cell signalling systems that are amenable to engineering into networks.

Ellington, Chen and co-workers have 'transformed' the task of generating chemical patterns by rational design. It remains to be seen whether these networks can react in response to other kinds of stimuli besides light (such as other molecules), and what kinds of process they will be able to control with their outputs. ❐

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ORIGIN OF LIFE

Cold-hearted RNA heats up life

An RNA replicase ribozyme has long been sought by chemists interested in the origin of life. Now, a selection strategy employing a low-temperature water–ice mixture as the medium has led to discovery of a ribozyme that can catalyse polymerization of an RNA chain greater than its own length.

Niles Lehman

The RNA world concept — that on the early Earth, RNA performed the roles now filled by enzymes and DNA, as both catalysts and information stores, respectively — relies on the existence of some mechanism for RNA molecules to make more of themselves without the aid of cellular machinery[1]. One obvious way that this could have happened was for RNAs to perform a function analogous to the modern enzyme RNA replicase: use an RNA template to guide the synthesis of a complementary strand, nucleotide-by-nucleotide. Since the 1980s the search has been on for an RNA enzyme (ribozyme) that can do just that. These efforts have been hindered by several factors, especially processivity. Ribozyme sequences that have been thrown at the problem are able to polymerize a dozen or so nucleotides but then tend to stop working[2–4].

A research team led by Philipp Holliger has had the most success of late, and in 2011 coaxed a version of the class I ligase ribozyme[5] to polymerize up to 95 nucleotides[6]. As impressive as this is, it still represented a product that was only half the length of the ribozyme itself. For nascent genes in the RNA world to have "unlimited heritability"[7] an RNA replicase ribozyme would have to generate products at least as long as itself. As now reported in *Nature Chemistry*, the Holliger lab has finally achieved this important milestone[8].

The winning strategy proved to be to search at low temperatures. Holliger and co-workers took advantage of an observation that the eutectic phase in

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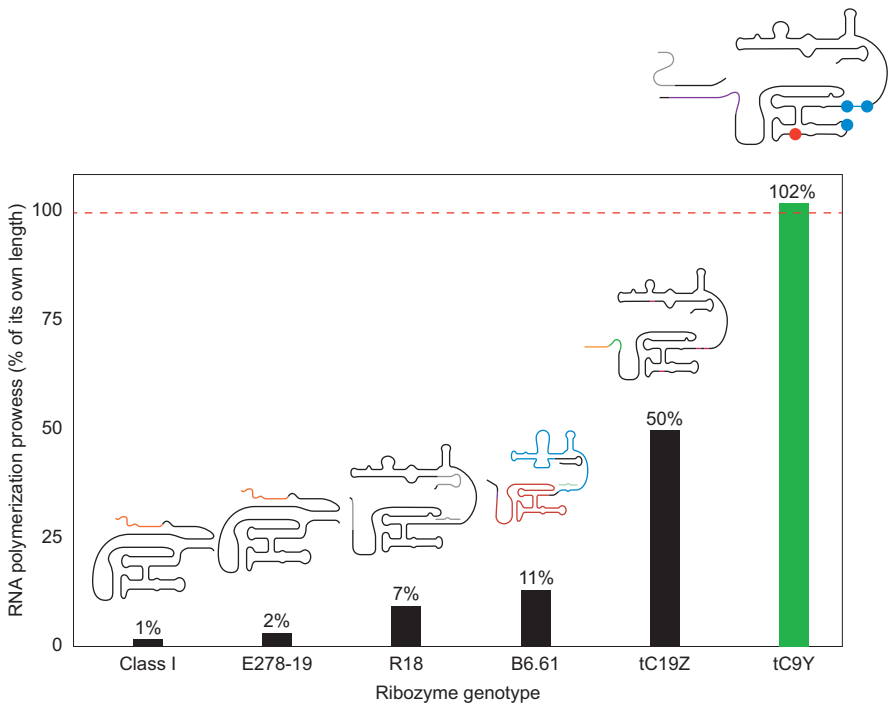

Figure 1 | Progress towards an RNA replicase ribozyme. The bar chart depicts various improvements to the class I ligase ribozyme[5] that can perform a single ligation reaction compared with its approximately 118-nucleotide length. Variants of this ribozyme type were selected *in vitro* to perform multiple ligation reactions in search of processive polymerization. These included the E278-19 ribozyme[3] that can perform three successive ligation reactions, the R18 ribozyme that can polymerize 14 nucleotides[2], the B6.61 ribozyme that can polymerize 20 nucleotides[4], and the tC19Z ribozyme, that can polymerize up to 95 nucleotides[6]. The latest development tC9Y, can polymerize up to 206 nucleotides[8], thereby finally exceeding its own length (202 nucleotides). Compared with tC19Z, this record-setting ribozyme has three cold-derived mutations (blue circles) in addition to one 'general up' mutation (red circle). The per-position error rates of most of these polymerases falls in the 0.1–1% range, putting them near the minimum level needed to sustain biological information in the long run.

a heterogeneous water–ice mixture enhances the polymerization yield of ligase ribozymes[9]. They then paired this result with a bead-based selection strategy: they created a library of microbeads, each bearing ribozymes with subtly varied sequences, and asked: which RNA can best extend a primer in the cold (–7 °C)? The answer, after a few rounds of in-ice selection and exhaustive genotyping, was a ribozyme differing by just four nucleotides from one of the well-known ligases. The original genotype, called R18, had been capable of polymerizing fairly reliably on the order of 14 nucleotides on a template. But the mutations in the new variant, Y, enhance both its processivity and its tolerance to low temperatures. Combining these four mutations with a previously discovered 5′ extension sequence that had been shown to promote long syntheses[6], results in the sequence called tC9Y. This genotype benefits from all known enhancements cobbled together so far, and holds the current world record for RNA-directed RNA polymerization at an astonishing 206 nucleotides (Fig. 1).

Low temperatures do the trick because RNA polymerization is basically an iterative ligation reaction, where the catalytic power of the ribozyme is required both to hold the nucleotide triphosphate substrates in place, and to facilitate the attack of the 3′-OH nucleophile at the end of the nascent RNA towards its target nucleotide triphosphate monomer. Whereas the latter activity enjoys a rate enhancement that should follow Arrhenius behaviour and improve with temperature, the former activity depends on successful binding of a substrate for long enough to allow the nucleophilic attack to take place. Here, the substrate and the template are both negatively charged, as is the catalyst, exacerbating the problem. All players in fact are RNAs, explaining why high concentrations of divalent cation — 200 mM Mg^{2+} — are also required to help the chemistry of polymerization. As a consequence, a temperature that is as low as possible while still maintaining a solution state strikes the balance between the kinetics of polymerization and the thermodynamics of binding. Holliger and co-workers also make the case that while cold temperatures play a significant role in this regard, the biphasic nature (water–ice) of the system may also be critical. Many other catalytic problems, including those in the prebiotic chemistry field, have been overcome by the employment of some sort of interface, such as lipid/solvent, surface/solvent or solvent/atmosphere. Using bulk solutions is a good place to begin an exploration of the novel chemistry that life brought to the Earth, but, given the likely complexity and tumultuousness of the planet some four billion years ago, moving to more heterogeneous environments may be required to achieve the kind of breakthroughs that the Holliger lab has achieved.

At first glance the improvement in polymerization prowess from 95 to 206 nucleotides may seem notable but hardly cause for excitement. After all, this represents little more than a two-fold gain. However, including its 5′ extension sequence, the tC9Y ribozyme itself is 202 nucleotides in length, meaning that this two-fold gain demonstrates the proof-of-principle that a polymerase ribozyme can copy a sequence of its own length. That said, tC9Y is only able to replicate certain sequences up to this length — indeed, it cannot replicate its own sequence.

Given the history of this ribozyme — from the first class I ligase selected from a random pool of sequences[5] to the first crude polymerase that could eke out a 12 nucleotide sequence[2], to far more robust variants discovered in the Unrau lab[4], to the C60U mutation that helped reach the 95 nucleotide extension mark[6], and the latest breakthrough[8] – it is certain that this highly artificial and engineered construct has little relationship to any historical RNA. In fact, no strong connection has been made from any natural ribozyme to RNA polymerase function. But these realities make the current tC9Y ribozyme that much more astonishing because its originating sequence did not have billions of years of evolutionary tinkering to give it a head start. Effectively, the tC9Y ribozyme has been painted into a corner with little room to grow. This implies that natural selection, which has proved time and again to be far more potent at creating activity than a few years of efforts in the lab, may well have crafted a fully processive and fully universal RNA replicase ribozyme. With tC9Y the latest prize added to the RNA world trophy cabinet, the likelihood that such an RNA existed at some point becomes far greater.

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Our vision of prebiotic Earth is continuing to warm up to the RNA world idea. But it might have actually been a cold fissure in a rock somewhere that generated the first spark of life. The discovery of tC9Y, a ribozyme that can polymerize sequences greater than its own length, inspires us to use similar strategies to reveal the replicative systems that must have preceded, and then descended from, an RNA replicase ribozyme[10]. ❐

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INTERMOLECULAR FORCES

A solution to dispersion interactions

London dispersion forces have been cited as an important factor in protein folding, drug–receptor interactions, and catalyst selectivities. However, careful analysis of a model system finds that the dispersion interactions are only minor contributors to the formation of complexes in solution.

Ken D. Shimizu

We often take for granted that molecules can form liquids and solids. Molecules are wrapped in a negatively charged cloud of electrons that should repel each other and inhibit the formation of condensed phases (Fig. 1a). Fortunately, all molecules can form attractive electrostatic interactions called London dispersion forces (named after the physicist Fritz London rather than the city of the same name). Even nonpolar molecules can form temporary instantaneous dipoles as a result of random asymmetries in their electron distributions (Fig. 1b). These temporary dipoles trigger the formation of complementary 'induced' dipoles in adjacent molecules (Fig. 1c). The electrostatic attraction between the instantaneous and induced dipoles is the basis for weak London dispersion forces.

Despite (or perhaps because of) their ubiquity, a key question is how much do dispersion interactions contribute to the association of solute molecules in solution (Fig. 2)? The answer to this question is important in understanding and optimizing many important processes that occur in solution, such as the selectivity of synthetic catalysts, the strength of receptor–drug interactions, and the fidelity of base pairing in DNA. Now, writing in *Nature Chemistry*, Scott Cockroft and co-workers tackle this interesting question[1]. They find that the net influence of London dispersion forces on solute–solute interactions in a wide range of different solvent systems is very weak to negligible.

Cockroft and colleagues focused on a small-molecule model system based on Wilcox's torsional molecular balance[2]. Using a scaffold derived from Tröger's base, this model system equilibrates between two distinct conformations in which two alkyl groups (one hexyl, one heptyl) are either aligned next to one another or held 180° apart. The intramolecular interactions between the two precisely positioned alkyl groups are formed and broken as the molecule switches from one conformer to the other. Thus, measurement of the conformational equilibrium provides a very sensitive measure (±0.12 kJ mol^{-1}) of the strength of the intramolecular interactions between the alkyl chains. The choice of Wilcox's system is very fitting, because previous analyses of this system have stimulated much of the conversation of the importance of dispersion interactions in solution[3–5].

The major difference in this particular study is the aim of trying to distinguish the very weak solute–solute dispersion effects from the omnipresent solvation or solvophobic effects[6]. The dispersion interactions were isolated by the combination of: (1) the study of the molecular balance in 31 different solvent environments spanning organic, fluorous, and aqueous solvent systems; (2) comparison of the data with three control balances to remove other potential influences of the molecular scaffold on the

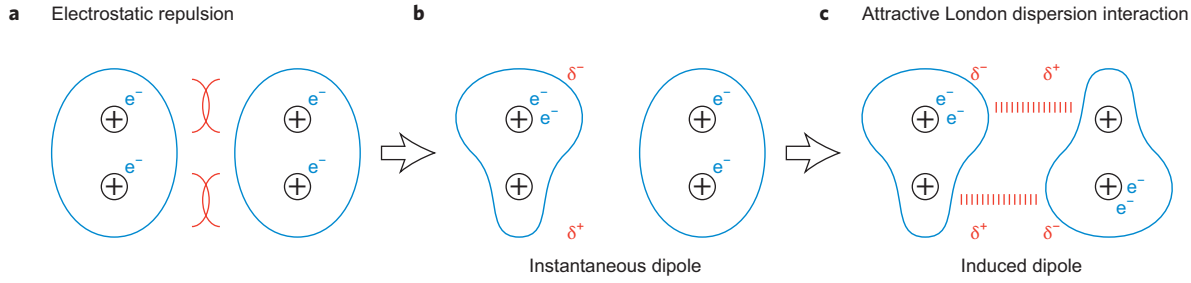

Figure 1 | Electrostatic origins of attractive intermolecular dispersion interactions. **a**, In molecules without electron mobility, there would be an electrostatic repulsion between electron clouds. **b**, The electron clouds are dynamic, however, and this leads to asymmetric electron distributions that give rise to instantaneous dipoles. **c**, The dipoles, in turn, induce the opposite asymmetric electron distribution resulting in attractive dispersion interactions known as London forces.

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