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Reflections of a Darwinian Engineer

Gerald F. Joyce[1]

[1]Department of Chemistry and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

This year marks the 25th anniversary of what is often regarded as a breakthrough year for in vitro evolution. In 1990, there was a confluence of biotechnological advances and deeper appreciation for the functional capacity of RNA that emboldened scientists to launch expeditions into the uncharted waters of RNA sequence space. Inspired by the power of Darwinian evolution in nature, as well as the pioneering experiments of Sol Spiegelman regarding the in vitro evolution of Qβ bacteriophage genomic RNA (Mills et al. 1967), it seemed that if one provided sufficient molecular diversity, selection stringency, and amplification horsepower, it might be possible to "breed" RNA molecules with user-specified properties. Inspiration also came from the notion of an "RNA world" (Gilbert 1986), a presumed time in the early history of life on Earth when RNA served as both the genetic material and the chief agent of catalytic function. It was too ambitious (although often motivational) to think about constructing an RNA-based life form in the laboratory, but a more tangible goal was to capture a piece of the RNA world by evolving novel functional RNA molecules.

For me there was another inspiration, which came a decade before the invention of PCR amplification and the discovery of catalytic RNA. In a story I have told previously (Joyce 1999), I was strongly affected by the novels of Thomas Pynchon, in particular *Gravity's Rainbow* (Pynchon 1973), which spoke allegorically of the universe's inexorable decay toward a state of maximum entropy and of human behavior swept along by the tide of physical laws. Yet Pynchon also pointed to what he called the "counterforce" or the "green uprising", which pushes in the opposite direction toward increased order and novel organization. Surely the counterforce on Earth is Darwinian evolution. Early twentieth-century physicists harnessed the "force" by splitting the atom, and perhaps late twentieth-century biologists could harness the counterforce by taming molecular evolution. Great technical advances on a massive scale were required to exploit atomic energy, whereas for Darwinian evolution one might be able to draw upon gadgets from biology and operate in volumes of less than a milliliter.

The devil was in the details, and it all came down to devising methods for the amplification, mutation, and selection of functional RNAs (Joyce 1989). PCR amplification of DNA was new at the time and the only method for RNA amplification involved Qβ replicase or other

✉Gerald F. Joyce, gjoyce@scripps.edu.

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RNA-dependent RNA polymerases. The problem with these polymerases is that, although advantageous in their biological context, they are specific for particular RNAs and cannot be used to amplify RNA in a sequence-general manner. The alternative was either to reverse transcribe the RNA to DNA and carry out PCR amplification or to use a combination of reverse and forward transcription to amplify the RNA directly. I preferred the latter approach because it could be carried out isothermally and thus would open the door for the eventual development of continuous in vitro evolution systems (Wright and Joyce 1997). Mutation was less of a challenge because polymerases were known to be error-prone, especially under modified reaction conditions (Kunkel et al. 1983; Shearman and Loeb 1983), and because combinatorial site-directed mutagenesis techniques had already been described (Wells et al. 1985; Oliphant et al. 1986). Selection seemed like the biggest challenge because it would require sensitive and specific methods for culling rare functional variants from vast, heterogeneous populations of RNAs.

I was fortunate to be invited to attend the 1987 Cold Spring Harbor Symposium, which that year was on the topic “Evolution of Catalytic Function”. Disappointingly, Thomas Pynchon was not an invited speaker, but there were many of the luminaries of RNA biochemistry, enzymology, and molecular evolution. Also disappointingly, nearly everyone at the meeting spoke about the evolution of catalytic function in the past tense, as if the world were already dead. But I knew the green uprising was still alive and waiting to be unleashed by technology. Walking along the shoreline at night and consulting with experts during the day, I was able to piece together a research plan for the in vitro evolution of group I ribozymes. Olke Uhlenbeck provided answers regarding how T7 RNA polymerase might be used to complete an isothermal amplification cycle. Jeremy Knowles gave insights into generating populations of random variants of (protein) enzymes. Tom Cech explained how the group I ribozyme could be made to perform splicing-related reactions on separate RNA substrates.

As a postdoctoral fellow I began to lay the groundwork for carrying out the in vitro evolution of RNA enzymes, but it was not until nearly 2 years later when I started my own laboratory at The Scripps Research Institute that I could pursue this goal in earnest. Isothermal amplification of RNA went much better than expected, enabling million-fold amplification in two hours (as first reported by Guatelli et al. 1990). Mutagenesis diverged into two techniques, one for generating complex starting libraries of RNAs (Joyce and Inoue 1989), and the other for introducing random mutations on the fly by diverting the cDNA component of the amplification mixture to a mutagenic PCR procedure (Cadwell and Joyce 1992). Selection also turned out to be less difficult than expected because the products of a chemical transformation can easily be separated from unreacted materials if the former contain a distinguishing chemical tag. The tag that we used initially was a portion of an oligonucleotide substrate that got transferred to the 3′-end of the ribozyme and served as a primer binding site for selective reverse transcription (Robertson and Joyce 1990). Oligonucleotide tagging is still popular today, enabling selection schemes based on primer binding, oligonucleotide hybridization, and altered gel-shift mobility. Biotin tagging has been the most popular selection method over the years, although alkyne tagging and subsequent capture by “click” chemistry is a rising trend.

1990 is generally considered to be the birth year for the in vitro evolution of RNA (Robertson and Joyce 1990; Tuerk and Gold 1990; Ellington and Szostak 1990), but in my view it was not until 1992–1993 that the technology became fully realized. By that time selective amplification and mutation had become fully integrated to enable true Darwinian evolution (Beaudry and Joyce 1992), completely novel RNA enzymes had been evolved starting from a population of random-sequence RNAs (Bartel and Szostak 1993), and RNA and DNA aptamers were obtained which bind ligands that do not normally contact nucleic acids (Bock et al. 1992; Famulok and Szostak 1992; Connell et al. 1993; Jellinek et al. 1993). Since the early 1990s the directed evolution approach has been used to analyze the features of hundreds of biological RNAs and to invent thousands of novel functional RNA and DNA molecules. The same techniques also have been applied to nucleic acid analogs with base modifications that enhance chemical functionality (Tarasow et al. 1997), sugar modifications that confer resistance to nuclease degradation (Lin et al. 1994), and wholesale changes to the sugar-phosphate backbone that move into the realm of “xeno” nucleic acids (Pinheiro et al. 2012; Yu et al. 2012).

The in vitro selection and in vitro evolution of nucleic acids is now such a standard technique that its inventive power is often taken for granted. Literally one begins with a population of random-sequence molecules and creates order in the form of a particular sequence composition that provides novel function. This is the counterforce. The power of Darwinian evolution in nature often is taken for granted too because the tendency is to focus on the products rather than the processes of evolution. Two recent developments, however, are changing that point of view. First, next generation sequencing technology has made it possible to follow the course of evolution, both directed and natural, in unprecedented detail. One can now connect the dots between molecules that are related by descent. Second, evolution can be seen to occur on the human timescale not just in directed evolution experiments, but also in the maturation of the adaptive immune response, the evolution of viral and microbial resistance, and the progression of cancer to a more undifferentiated state.

For Darwinian engineers such as myself, the fun has always been to watch evolution in motion, to see the bar keep rising for a new phenotype, and to catch the first glimpse of sequence data from an evolved population. I am currently interested in using directed evolution to study how natural RNA, composed of D-nucleotides, interacts with non-natural enantiomeric RNA, composed of L-nucleotides. RNA molecules of opposing handedness cannot form consecutive Watson–Crick pairs (Garbesi et al. 1993), so cross-chiral interactions must take place exclusively through tertiary structural contacts. It is not clear whether biological evolution ever explored this mode of interaction, but in vitro evolution provides a means to do so. Cross-chiral aptamers and enzymes are out there to be found (Sczepanski and Joyce 2013, 2014).

The ultimate goal for a Darwinian engineer is to devise an evolving system that can evolve on its own. There are reasons to believe this may be possible for RNA, based not just on consideration of the presumed RNA world, but also on growing understanding of the catalytic potential of RNA. The class I ligase ribozyme, the first enzyme to be obtained starting from random-sequence molecules (Bartel and Szostak 1993), was subsequently evolved to function as an RNA-dependent RNA polymerase (Ekland and Bartel 1996) and

then to become a much more robust form of that polymerase (Wochner et al. 2011). I am confident there will be further dramatic improvements in the activity of this enzyme, including the ability to function as an RNA replicase for amplifying short RNA substrates. There is one reported case of an in vitro evolved RNA enzyme that catalyzes its own replication and can undergo exponential amplification, enabling self-sustained Darwinian evolution (Lincoln and Joyce 2009). However, that enzyme has very limited capacity for the invention of novel function. The field still awaits the development of a general-purpose RNA replicase ribozyme.

The past quarter century has truly been remarkable, bountiful in the development of directed evolution technologies and their application to both basic scientific questions and practical aims. The universe as a whole may be streaming toward a state of maximum entropy, but we can create local pockets of order from randomness. We can prepare tiny volumes of liquid that contain trillions of random-sequence RNAs and drive those molecules through repeated cycles of amplification, mutation, and selection to obtain the fruits of Darwinian innovation. For scientists who have shared in the past 25 years of excitement, and for those who wish to contribute over the next 25 years, there are many interesting opportunities waiting in sequence space. Just think what you might find!

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References

Bartel DP, Szostak JW. Isolation of new ribozymes from a large pool of random sequences. Science. 1993; 261:1411–1417. [PubMed: 7690155]

Beaudry AA, Joyce GF. Directed evolution of an RNA enzyme. Science. 1992; 257:635–641. [PubMed: 1496376]

Bock LC, Griffin LC, Latham JA, Vermaas EH, Toole JJ. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. Nature. 1992; 355:564–566. [PubMed: 1741036]

Cadwell RC, Joyce GF. Randomization of genes by PCR mutagenesis. PCR Methods Appl. 1992; 2:28–33. [PubMed: 1490172]

Connell GJ, Illangesekare M, Yarus M. Three small ribooligonucleotides with specific arginine sites. Biochemistry. 1993; 32:5497–5502. [PubMed: 8504070]

Ekland EH, Bartel DP. RNA-catalysed RNA polymerization using nucleoside triphosphates. Nature. 1996; 382:373–376. [PubMed: 8684470]

Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990; 346:818–822. [PubMed: 1697402]

Famulok M, Szostak JW. Stereospecific recognition of tryptophan agarose by in vitro selected RNA. J Am Chem Soc. 1992; 114:3990–3991.

Garbesi A, Capobianco ML, Colonna FP, Tondelli L, Arcamone F, Manzini G, Hilbers CW, Aelen JM, Blommers MJ. L-DNAs as potent antimessenger oligonucleotides: a reassessment. Nucleic Acids Res. 1993; 21:4159–4165. [PubMed: 8414968]

Gilbert W. The RNA world. Nature. 1986; 319:618.

Guatelli JC, Whitfield KM, Kwoh DY, Barringer KJ, Richman DD, Gingeras TR. Isothermal, in vitro amplification of nucleic acids by a multienzyme reaction modeled after retroviral replication. Proc Natl Acad Sci USA. 1990; 87:1874–1878. [PubMed: 2308948]

Jellinek D, Lynott CK, Rifkin DB, Janjić N. High-affinity RNA ligands to basic fibroblast growth factor inhibit receptor binding. Proc Natl Acad Sci USA. 1993; 90:11227–11231. [PubMed: 7504300]

Joyce GF. Amplification, mutation, and selection of catalytic RNA. Gene. 1989; 82:83–87. [PubMed: 2684778]

Joyce GF. The counterforce. Curr Biol. 1999; 9:R500–R501. [PubMed: 10421584]

Joyce GF, Inoue T. A novel technique for the rapid preparation of mutant RNAs. Nucleic Acids Res. 1989; 17:711–722. [PubMed: 2464798]

Kunkel TA, Schaaper RM, Loeb LA. Depurination-induced infidelity of deoxyribonucleic acid synthesis with purified deoxyribonucleic acid replication proteins in vitro. Biochemistry. 1983; 22:2378–2384. [PubMed: 6344919]

Lin Y, Qiu Q, Gill SC, Jayasena SD. Modified RNA sequence pools in in vitro selection. Nucleic Acids Res. 1994; 22:5229–5234. [PubMed: 7529404]

Lincoln TA, Joyce GF. Self-sustained replication of an RNA enzyme. Science. 2009; 323:1229–1232. [PubMed: 19131595]

Mills DR, Peterson RL, Spiegelman S. An extracellular Darwinian experiment with a self-duplicating nucleic acid molecule. Proc Natl Acad Sci USA. 1967; 58:217–224. [PubMed: 5231602]

Oliphant AR, Nussbaum AL, Struhl K. Cloning of random-sequence oligodeoxynucleotides. Gene. 1986; 44:177–183. [PubMed: 3023181]

Pinheiro V, Taylor A, Cozens C, Abramov M, Renders M, Zhang S, Chaput JC, Wengel J, Peak-Chew S, McLaughlin S, Herdewijn P, Holliger P. Synthetic genetic polymers capable of heredity and evolution. Science. 2012; 336:341–344. [PubMed: 22517858]

Pynchon, T. Gravity's rainbow. New York: Viking Press; 1973.

Robertson DL, Joyce GF. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature. 1990; 344:467–468. [PubMed: 1690861]

Sczepanski JT, Joyce GF. Binding of a structured D-RNA molecule by an L-RNA aptamer. J Am Chem Soc. 2013; 35:13290–13293.

Sczepanski JT, Joyce GF. A cross-chiral RNA polymerase ribozyme. Nature. 2014; 515:440–442. [PubMed: 25363769]

Shearman CW, Loeb LA. On the fidelity of DNA replication: specificity of nucleotide substitution by intercalating agents. J Biol Chem. 1983; 258:4477–4484. [PubMed: 6339499]

Tarasow TM, Tarasow SL, Eaton BE. RNA-catalysed carbon–carbon bond formation. Nature. 1997; 389:54–57. [PubMed: 9288965]

Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990; 249:505–510. [PubMed: 2200121]

Wells JA, Vasser M, Powers DB. Cassette mutagenesis: an efficient method for generation of multiple mutations at defined sites. Gene. 1985; 34:315–323. [PubMed: 3891521]

Wochner A, Attwater J, Coulson A, Holliger P. Ribozyme-catalyzed transcription of an active ribozyme. Science. 2011; 332:209–212. [PubMed: 21474753]

Wright MC, Joyce GF. Continuous in vitro evolution of catalytic function. Science. 1997; 276:614–617. [PubMed: 9110984]

Yu H, Zhang S, Chaput JC. Darwinian evolution of an alternative genetic system provides support for TNA as an RNA progenitor. Nat Chem. 2012; 4:183–187. [PubMed: 22354431]