A step through the looking glass

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Received June 8, 2016; accepted June 15, 2016; published online June 24, 2016

Citation: Liang, Y., Ang, E.L., and Zhao, H. (2016). A step through the looking glass. *Sci China Life Sci* 59, 979–980. doi: 10.1007/s11427-016-0170-6

Despite our many advances in deconstructing and understanding cellular and biomolecular machineries, the homochirality of life remains an enigma. Since our earliest ancestors, the central dogma has operated solely on D-DNA and L-amino acids, even though the laws of physics and chemistry do not actually dictate any preference over the mirror-image counterparts. This conundrum has led some inquiring minds to ask the question if chirally-inverted versions of transcription and translation were possible, and to envision an alternate life form based on mirror-image L-DNA and D-proteins. To address this, a team at Tsinghua University recently constructed a synthetic D-DNA polymerase entirely from D-amino acids and put the theory of mirror-image transcription to the test (Wang et al., 2016).

In this work, the team used peptide synthesis and chemical ligation, as well as a few tricks of the trade, to generate a 174 residue-African swine fever virus (ASFV) polymerase (the smallest known DNA polymerase) entirely from D-amino acids, and further demonstrated that the synthetic D-ASFV polymerase was able to carry out mirror-image transcription by polymerizing L-DNA, albeit with very low efficiency (it took four hours to extend a 12-nucleotide primer to 18 nucleotides). The low efficiency of the polymerase was attributed to the inherent nature of the small ASFV rather than any defects of the synthetic mirror-image polymerase. The team further showed that the synthetic D-polymerase exhibited chiral specificity and was unable to process DNA templates, primers or dNTP of the natural-

ly-occurring D-form. There was also no observable enantiomeric cross-inhibition when L- and D-enzymes and their corresponding substrates were pooled in a racemic mixture. In addition, it was proven for the first time that both the native and synthetic mirror-image ASFV polymerases were able to polymerize RNA using DNA templates, although at much lower efficiencies and specificities than the native DNA polymerization reactions.

While being a proof-of-concept study, this landmark work provides an attractive alternative to the relatively inaccurate and size-limiting chemical route currently used for production of L-nucleic acids, and opens up exciting possibilities for the enzymatic production of L-DNA and L-RNA through polymerase chain reaction and *in vitro* transcription. For these envisioned applications to be realized, the synthetic D-ASFV polymerase needs to be improved for better thermo-tolerance and much higher enzymatic efficiencies. Like any other protein, protein engineering can be carried out for the synthetic D-ASFV to generate the desired enhanced variants. Alternatively, mirror-image versions of other more efficient and thermo-tolerant polymerases can be created using a synthetic approach similar to the one adopted by the authors.

More excitingly, the mirror-image transcription machinery described in the paper represents a momentous first step towards the construction of a mirror-image central dogma; and ultimately, mirror-image life. The next major hurdle that stands in the way of this vision is the development of a translation machinery capable of churning out D-peptides and proteins. Toward this goal, synthetic biologist, George Church, has been trying to generate mutant ribosomes

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which are capable of translating regular D-RNA transcripts into D-peptides, albeit with limited success (Bohannon, 2010; Brockman, 2007; Singer, 2009). As an alternative to the mutagenesis approach, which involves the creation and screening of huge mutant libraries, the Tsinghua team proposed the complete chemical synthesis of small ribosomal protein units from D-amino acids followed by their *in vitro* assembly into functional D-ribosomes. With their latest feat and the previously demonstrated *in vitro* assembly of synthetic ribosomes (Jewett et al., 2013), the proposed synthetic route to a mirror-image translation machinery may have greater chances of success than ever. However, the realization of this dream is still contingent on further advances in peptide synthesis for the generation of longer and purer peptides. Once the initial and greatest hurdle of a mirror-image translation machinery has been crossed, the progress towards the construction of a mirror-image cell should be exponential as the other required components such as helicases and ligases can be generated enzymatically rather than through the laborious route of chemical synthesis.

With the numerous possibilities opened by this study, the potential and dangers of mirror-image life are brought into the spotlight once again. The enhanced ability to manipulate molecules on the other side of the mirror would be extremely useful for production of chiral-specific pharmaceuticals, as well as therapeutic, degradation-resistant D-peptides, L-DNA and L-RNA aptamers. Mirror-image cells will also serve as invaluable models to address fundamental biological questions such as the reasons behind the homochi-

rality of life. With its inherent incompatibility with life on earth, synthetic mirror-image cells will also be resistant to viruses and naturally-occurring enzymes and inhibitors. This competitive advantage will make the cells very attractive production hosts for industrial bioproduction. However, with the absence of competition, predators, and diseases to limit the proliferation of these synthetic cells, tight regulation and safeguards have to be put in place to forestall the genetic engineering-turned-doomsday scenario favored by Hollywood. All in all, this work represents an exciting first step towards the realm of mirror-image life and we should be seeing a lot more progress in this area in the near future.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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