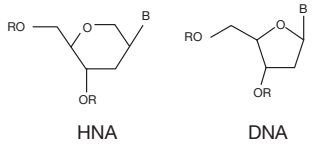
SYNTHETIC BIOLOGY

Understudies of DNA and RNA

With the help of engineered polymerases and reverse transcriptases, synthetic nucleic acids can encode and pass on genetic information.



Schematic comparing the sugar in hydrohexitol nucleic acid (HNA) and DNA. B, nucleobase; R, phosphate backbone.

The fact that in biology information flows bidirectionally—from DNA to RNA and then back to DNA—raised a question for Phil Holliger at the Medical Research Council (Cambridge, UK): is there something inherently special about DNA and RNA, or can other polymers exchange information as well?

In the last 30 years, biological chemists have developed a wide range of potential DNA mimics—which Holliger refers to as xeno-nucleic acids (XNA)—that have the ability to hybridize to DNA and RNA: for example, locked nucleic acids, which are widely used as inhibitors of mRNA or microRNAs. But to answer his questions, Holliger needed to go beyond hybridization. "We wanted not just to synthesize polymers but [to] create a replication loop, from DNA to XNA back to DNA," he says.

His team decided to concentrate on changing the sugar in the nucleic acid rather than the phosphate backbone or the nucleobases. Because they retained the classical nucleobases G, A, T and C, the XNAs also kept the ability to interact with DNA. The researchers swapped the canonical five-membered ribofuranose sugar ring for other structures—for example, hydrohexitol to make hydrohexitol nucleic acids (HNAs).

To establish heredity, the team needed a system that allows replication. They evolved a polymerase capable of transcribing DNA into XNA using compartmentalized self-tagging (CST) to select for suitable candidates. In CST, a pool of randomly mutagenized polymerases, compartmentalized in an oil-and-water emulsion together with primers, XNAs and a plasmid

CELL BIOLOGY

Refined siRNA SCREENS

Bioinformatic discrimination of siRNAs that perturb cells via direct versus indirect effects can improve data quality and change hit lists in image-based screens.

Cells growing in a culture dish are not uniform entities; they vary both stochastically and as a consequence of external signals. One source of external variability is the microenvironment of the cells, and as Lucas Pelkmans, Berend Snijder and their colleagues at the University of Zurich have previously shown, microenvironmental properties such as cell density—what they call 'population context effects'—can strongly affect biological processes.

Such population effects could also influence phenotypes that result from a perturbation, for instanct, gene silencing by RNAi. Pelkmans and colleagues now systematically analyze population context effects on siRNA screens in cells and describe methods to understand and counter these effects (Snijder *et al.*, 2012).

The researchers began with image data from 34 small-scale (about 50 genes) and 7 large-scale (about 7,000 genes) siRNA screens that examined the infection of four different HeLa cell 'strains' with 17 viruses. As in their previous work, they observed that population context can affect the levels of viral infection. They also found that a model of this relationship could predict a large portion of the measured viral infection levels following siRNA treatment. This indicates that some phenotypes in the analyzed screens were in fact due to indirect effects of the siRNA on the cells. "To put it simply," explains Pelkmans, "if you have a virus that prefers to infect very densely growing cells, and you now silence a gene that reduces cellular growth, you get a population where there are less cells in the right state for infection, and the model can predict that." Pelkmans emphasizes, however, that population effects are complex and cannot be predicted by the number of cells alone.

In the case of siRNA treatments for which the model fails to fully explain the phenotype, the siRNA is likely to perturb viral infection via direct cellular effects. Following this reasoning, Pelkmans and colleagues used their model to distinguish

encoding the polymerase tries to extend the primer; only an active polymerase will be able to do so, allowing capture and enrichment of the complex. In Hollinger's experiment, CST readily selected polymerases that worked with HNAs.

Polymerases in hand, Holliger wanted to close the loop and copy XNAs back to DNA. Not only would this permit more accurate analysis of the replication process (by providing a means of estimating error rate), but it would also allow evolution of the sequences. Unfortunately, no polymerases displayed XNA reverse trancriptase (RT) activity, so the researchers engineered an RT *de novo* that could translate the information encoded in the HNA back into DNA. It did not do so perfectly, nor did the polymerase transcribe with high fidelity: the error rates of replication and RT ranged from 5 in 100 to 4 in 1,000.

Holliger used the error rate to his advantage when exploring whether the HNA polymers could be subjected to evolution. His team sought to select HNA aptamers that specifically bound two targets. Starting with a diverse repertoire of HNAs, the scientists had to go through eight rounds of selection—enrichment of HNA oligos that bound to the respective motifs, followed by a round of replication—before seeing the consensus motifs substantially enriched.

Holliger sees the production of aptamers as one of the applications for these polymers. Current aptamers based on DNA and RNA are rapidly degraded if they are not chemically fortified. Not so for HNAs: they are resistant to degradation and sufficiently orthogonal to natural nucleic acids so as to not be toxic. "For me this provides a glimpse of what will be possible," Holliger says. "It will be the beginning of ever-advanc[ing] chemistry that will allow highly desirable properties for all sorts of applications."

Applications aside, what this work has shown clearly is that several polymers have the capacity for heredity and evolution. Holliger says, "In the relation to the origin of life, it shows that the choice of RNA to get life started is largely based on opportunism."

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RESEARCH PAPERS

Pinheiro, V.B. *et al.* Synthetic genetic polymers capable of heredity and evolution. *Science* **336**, 341–344 (2012).

between siRNAs that cause a phenotype via direct versus indirect effects. They also used Bayesian modeling to learn the causal relationships that lead to the observed phenotypic changes.

What consequence does separating direct and indirect effects have on the outcome of siRNA screens? Most strikingly, for both small- and large-scale viral infection screens, the top-ranked genes changed substantially when hit lists were corrected to remove indirect, population-based effects. For infection by the SV40 virus, removing indirectly acting siRNAs yielded hit lists enriched for genes known to be involved in viral entry.

Correcting for indirect siRNA effects improved the overlap between screens performed on different cell lines and even, in some cases, in different laboratories; these improvements were noted for screens examining several biological properties (organelle abundance, cell size or cellular cholesterol levels) in addition to viral infection. Removing siRNAs with indirect effects also increased the consistency of phenotypes produced by multiple siRNAs targeting the same gene. Pelkmans and Snijder emphasize, however, that the main consequence of implementing their corrections is that the results and their ranking change for a given screen. "What this does is it basically focuses you on a different set of genes," says Pelkmans, "and as far as we can tell, it focuses much more on the direct regulators of the cell biology."

Considering that cellular heterogeneity is a widespread phenomenon and that correction for indirect effects improved data quality for several types of image-based screens, Pelkmans suggests that other scientists conducting siRNA screens should consider that population context could affect the processes they are studying as well. If so, methods such as those used in this work could help to refine the resulting gene lists and allow researchers to focus their follow-up efforts on those genes in which they have the most interest.

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RESEARCH PAPERS

Snijder, B. *et al.* Single-cell analysis of population context advances RNAi screening at multiple levels. *Mol. Syst. Biol.* **8**, 579 (2012).