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MILESTONE 5

A reverse proves to be an advance

The central dogma of molecular biology states that DNA makes RNA makes protein, yet one of the most important DNA technologies stemmed from the discovery that the first of these steps can be reversed.

In 1970, puzzled by the ability of RNA tumour viruses to stably transform cells — without incorporation of a DNA copy of viral genes into the host genome — Baltimore, and Temin and Mizutani, looked for DNA polymerase activity in purified preparations of such viruses. The kinetics of the incorporation of radiolabelled thymine indicated that DNA was being synthesized. The reaction was sensitive to ribonuclease treatment, showing that it was RNA-dependent, whereas the product was deoxyribonuclease-sensitive but ribonuclease-insensitive, confirming that it was DNA.

Two years later, it was demonstrated that the reverse transcriptase could be used *in vitro* to synthesize cDNA from mammalian mRNAs. Verma *et al.* and Kacian *et al.* both added preparations of globin mRNAs to reverse transcriptase from avian myeloblastosis virus. They correctly hypothesized that the reaction would only work efficiently if they also added oligo(dT), which would hybridize to the poly(A) tail of the mRNAs and act as a primer. By hybridizing their DNA product to the original mRNA template, they confirmed that they had successfully synthesized cDNA.

Over the next two decades, reverse transcriptase was widely used for the cloning of expressed genes. However, one of the biggest technical hurdles, especially for the creation of comprehensive libraries, was that most cDNAs in a given reaction were not full length owing to premature termination. In addition, low-abundance transcripts were far less likely to be cloned than high-abundance ones. In the late 1990s, Carninci, Hayashizaki and colleagues developed several techniques to overcome these problems.

By biotin capping of the mRNAs, they ensured that only full-length cDNAs were selected. After first-strand cDNA synthesis, RNAse I was used to destroy any part of any mRNA that was not bound to cDNA. This caused the removal of the 5′ biotin cap from the mRNAs of all non-complete cDNAs. Magnetic beads were used to select only the full-length cDNAs for second-strand synthesis and cloning.

Their discovery that trehalose makes reverse transcriptase more thermostable meant that the reaction could be carried out at a higher temperature, at which the formation of fewer RNA secondary structures increased the number of full-length cDNAs produced.

Finally, in order to complete expression libraries, they selectively cloned rare new cDNAs by screening out abundant ones and those already cloned in existing libraries. To achieve this, they added biotinylated RNA from the original sample and existing libraries after first-strand synthesis. All of the cDNA that hybridized to the RNA was removed with magnetic beads, leaving rare cDNAs behind.

From its origin as an esoteric property of certain viruses, reverse transcription has become hugely important in molecular biology. Its influence extends from cloning to the development of microarrays to the annotation of genomes.

Patrick Goymer, Associate Editor,
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