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EDITORS' CHOICE

Science is a hard mistress

To do research is to have a series of incredible highs interspersed with often as many daunting lows. The highs arise from those infrequent discoveries that come after months if not years of grueling work in the lab when things suddenly fit together and a door to the future opens. The lows come from those times when you realize that the data you have obtained with all that work, just do not fit your favorite hypothesis and you must reject it. It seems paradoxical but the lows are what it is all about. Unlike any other field of human endeavor, scientific research demands a basic humility in the face of facts, a humility that flies in the face of human nature. Everyone wants to be respected and even famous and to reach a position of power and influence, and these human desires often lead to the avoidance of inconvenient facts either consciously or subconsciously. The researcher however must look at the data and interpret them in terms of the best model even if it means rejecting your own hypothesis.

I myself have experienced both types of emotions in my research career. The high came in 1990 when we suddenly realized that we could explain the extremely enigmatic and even disturbing phenomenon of uridine insertion/deletion RNA editing in trypanosomes that was causing serious scientists to speculate that the genetic dogma of information flow from DNA to RNA to protein was incomplete. We and others had found that multiple transcripts of the maxicircle mitochondrial DNA in trypanosome mitochondria could not be translated due to the absence of open reading frames, and that these transcripts were somehow corrected after transcription by the insertion and occasional deletion of uridine residues at specific sites thereby eliminating the encoded frame shifts and producing mRNAs with open reading frames that encoded conserved mitochondrial proteins. Initially the phenomenon was thought to involve a few U's at a few sites, but soon it blossomed into cases of hundreds of U's at hundreds of sites, in essence creating genes de novo.

The overriding question was where did the information come from that told the U's to be inserted and deleted at these precise sites. The information did not appear to be encoded anywhere in the mitochondrial genome, which was in itself quite bizarre in that it consisted of around 50 maxicircles catenated with thousands of minicircles into a single giant network of DNA. Not giving up on the central dogma we were looking for RNAs that could base pair with mature edited sequences and thereby provide the information by this well tested mechanism. We speculated that perhaps the reason no one had seen this sequence information was that it was in short segments. Having asked our friendly lab computer to search for short sequences anywhere in the maxicircle genome that could base pair with the mature edited sequences and thereby encode the insertions and deletions of U's, it was at first disappointing that there were no such antisense sequences. But a closer examination of the results showed that the mismatches were always transitions and suddenly we realized that if we took off our Watson Crick blindfolds and simply allowed G-U base pairs in addition to G-C and A-U base pairs, the computer was telling us that there were short complementary sequences in the maxicircle DNA that could encode the editing information. A few days later we obtained some direct evidence for the existence of a novel class of small RNAs with these sequences (and also with 3′ non-encoded oligo U tails!) and we named them "guide RNAs".

The next high came when we (i.e. Nancy Sturm, my graduate student) realized that the thousands of minicircles also actually encoded the majority of guide RNAs and that this was finally the long sought after solution to the genetic function of minicircles. Suddenly we had two mitochondrial genomes in the same cell, one with cryptogenes and another one with complementary guide RNA genes.

Immediately this discovery led to a mechanism in which the gRNAs formed an anchor duplex

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with the pre-edited mRNA just downstream of the editing site and recruited a cadre of specific enzymes to the editing site. We proposed a nuclease that cleaved the pre-edited mRNA at the editing site, a 3′ terminal uridylyltransferase that added U's or a 3′-5′ U-specific exonuclease that deleted U's, and finally an RNA ligase that religated the cleavage fragments. Like all good models it was very satisfying since it explained a number of previous observations such as the 3′—5′ polarity of editing. This was a definite high in the life of my lab.

But my Swiss postdoc, Beat Blum, had the habit of thinking too much and soon came up with another model — in which the U's were transferred from the known 3′ oligo U tail of the gRNAs to the editing sites by a transesterification mechanism such as employed in RNA splicing. This model had an easily testable prediction — that there were chimeric intermediates in which the 3′ end of the gRNA was covalently linked to the mRNA 3′ cleavage fragment at an editing site. When this was rapidly confirmed, the lab entered another high, albeit with a low level of anxiety and chagrin that we had just proposed another seemingly viable model and now were saying that this was wrong. The transesterification model became the flavor of the week and even the Nobel laureate, Tom Cech, independently proposed an identical hypothesis. The new vistas opened were awesome: Editing was now a type of RNA splicing and was a very ancient phenomenon indeed!

But then evidence slowly accumulated drip by drip that the chimeric intermediates were artifacts of cleavage ligation and that our original theory was correct and not the awesome transesterification model. Again a beautiful theory crashed on the hard rocks of inconvenient facts, but the inevitable low was tempered by the high that remained from the fact that our original model was indeed correct.

A few years have passed since those exciting days but the memories linger.

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