

Epigenetic Heredity: Transfer RNA Has Gone to Pieces

All eukaryotic (and many bacterial) RNAs descend from larger precursors, some of them thousands of times larger than the final product and others just a few nucleotides longer. The genomes of eukaryotes are pastiches of both assembled modules and subsequently introduced mobile elements, and the resulting baroque design has led to a situation in which almost all transcripts require trimming. The most dramatic case, of course, is the interruption of almost all eukaryotic transcription units for messenger RNA by intervening sequences, a.k.a. introns. Not the ideal plan, you say? Perhaps, but we cannot play the evolutionary tape backward to witness what forces were at play in the purifying selection that led to the adoption of these designs for the production of RNA. Adopted they were.

In growing mammalian cells, messenger RNAs (mRNAs) live for 6–24 hours on average, with some outliers on either side of that temporal window. The mRNA encoding the protein *c-myc* in mammalian cells lives for only a few minutes, whereas mRNAs produced during oogenesis in some animals are stored for months prior to the gamete's maturation. As regards ribosomal RNA, in many cells it is so stable that its lifetime cannot be measured. When RNAs have lived out their life (a time that appears to be stochastic for some, *i.e.*, the probability of an RNA's decay being unrelated to its posttranscriptional age, *vs.* others that are marked for a certain persistence) it has generally been thought that the degradation processes, while perhaps spatially directed to a degree (chewing in from either end or slicing at some hypervulnerable sites in the middle), would typically generate a Walpurgis Night of pieces. These have been conceptually assigned to a trash bin.

Alas, we now have a surprise. At least one class of mature (processed) RNAs, eventually going to pieces, produces some functional fragments.

CENTRAL DOXOLOGY: THE ADAPTOR “THAT HAD TO EXIST”

Transfer RNA (tRNA) was predicted by Francis Crick as the entity that would have to exist in order for amino acids to be installed on armatures that would confer upon each one its informational resonance with the gene, embodied as mRNA, thus closing the arms of the Central Dogma. This elegant prediction (I think it was his most brilliant of any, even though he thought these adaptors would need to be only 3 nt) was fulfilled by its discovery shortly after (1–3). Later work on tRNA focused on how each of the different amino acid-accepting species is read by the cognate enzyme, a field created and led by Paul Schimmel at MIT and later at Scripps (4). Into the 1980s and 1990s, all seemed halcyon about tRNA.

MicroRNAs, very different from tRNAs, were discovered in 1993 by Victor Ambros and Gary Ruvkun (5, 6).

These RNAs are a footprint of the ancient (anteprotein) RNA world, and they are major regulatory agents of gene expression at the level of protein synthesis. When the microRNA field was ablaze, as it continues to be, a few papers appeared reporting that tRNA fragments found in various cells seemed to have properties like those of microRNAs (reviewed in ref. 7). These findings were enabled by the advent of extremely sensitive deep sequencing methods for small RNAs. The idea that tRNAs could produce fragments with microRNA-like, *i.e.*, translation regulatory properties (or any other types of functions, for that matter) seemed to some in the field to be implausible (though reasons for this position were not given).

Two recent studies have now brought tRNA fragments into the limelight, this time with demonstrated functionality.

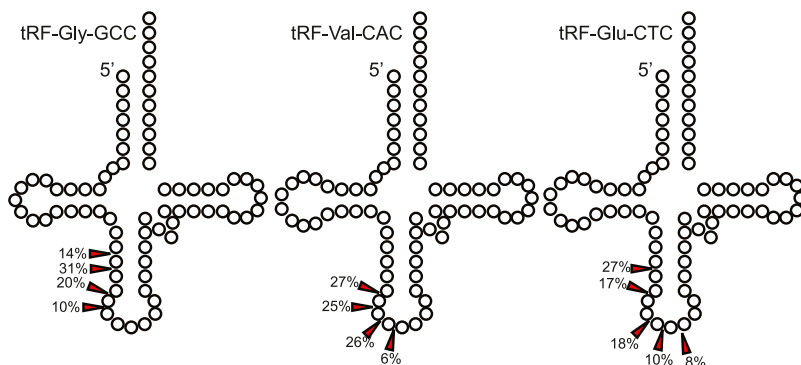
INHERITANCE OF ACQUIRED TRAITS

About nomenclature: what is to be described is often called “intergenerational inheritance.” This is glaringly incorrect, because inheritance is intergenerational by definition. Appending the adjective “epigenetic” makes the resulting term acceptable. But let us bear in mind that not all so-called epigenetic marks on chromosomes are heritable (8).

The notion that organisms can “acquire” a trait through some experience that a parent encountered may have floated about in antiquity but was brought to resonance by the French naturalist Jean-Baptiste Lamarck (1744–1829). Lost in the mist of history is the fact that Lamarck was an accomplished biologist and that his reasons for proposing this were not implausible as a hypothesis at the time. The British author Rudyard Kipling reinforced the notion through his “just so” stories, at least in the fanciful minds of some children (and perhaps the parents reading to them). Lamarck's views also played a role decades later, in the 1930s, when an ill-fated Soviet agricultural regime led to the demise of much of Russia's legitimate biologic science elite, stemming from an obsessive belief in the inheritance of acquired traits by Trofim Lysenko. By the mid-20th century, “Lamarckism” had become one of biology's dirtiest baskets of laundry, and it took great volumes of soapsuds to wash away the grime. But a funny thing happened: the idea, in fact, didn't go away.

Reports of disorders in children whose mothers had carried them *in utero* during the winter of 1944 and spring of 1945, when the Nazis had cut off food supplies to Holland alerted pediatricians and epidemiologists to the possibility that either parent's nutritional deprivation had been the cause, with obvious initial hypotheses

Figure 1. Sperm tRNA fragments implicated in epigenetic intergenerational inheritance. The arrows indicate the cleavage sites producing the 5' fragments implicated, with the abundance of the products indicated as percentages. From Sharma *et al* (9). Reproduced by permission of the American Association for the Advancement of Science.



centering on the intrauterine experience. Years later, this phenomenon was recapitulated in animal studies, and, in particular, descent from the paternal line was demonstrated (thus ruling out the intrauterine experience). Long-term postwar studies have tracked effects into an F2 generation. Meanwhile, additional animal studies confirmed that males encountering nutritional restriction produce “marked” sperm that carry an epigenetic memory and hand down effects into both the F1 and F2 generations.

SIC TRANSIT SPERMIA: THE EPIDIDYMIS AND tRNA

In two recent papers, this phenomenon has been reduced to molecular biology, and the agents (or at least one of the agents) in the pathway of genomic modification have been identified: fragments of tRNA. Oliver Rando and colleagues (9) had been intrigued about the male descent of “acquired” traits in mice, using the standard paradigm of nutritional limitation of the fathers. In their newest work, a tour de force of experimental design and understanding of testis biology, they assayed the epididymal fluid of the fathers and found the presence of tRNA fragments (Fig. 1) which additional experiments revealed to be the players in the epigenetic marking behind the epigenetic intergenerational inheritance (9). In a second elegant study, published in parallel, tRNA fragments were also implicated in a somewhat different experimental paradigm as regards the nutritional variable to which the fathers were subjected (10).

While the two meiotic divisions leading to spermatogonia and spermatids occur earlier in the mammalian testis, more lies ahead for these cells prior to ejaculation. The locus of these late transit events is the epididymis (Fig. 2) where signal transduction events install the maturing spermatid with its final credentials (11). Epididymal fluid can be collected from rats, hamsters, and mice, and in the hands of skillful investigators can even be collected from different locations within the epididymis, and studies on these fluids had informed a major axis of mammalian reproductive biology for decades. In 2012, a little-noticed publication reported the presence of tRNA fragments in mouse sperm (12), and 2 years later, tRNA fragments were reported as well in mouse semen, in extracellular vesicles (13).

These new findings (9, 10) are profound game changers. Clearly, much now needs to be done to understand exactly what these tRNA fragments are doing to set in motion a play-it-forward imprint of a male parental (or male grandparental) experience. The implicated tRNA fragments are from the 5' end, so there is no likelihood that they carry an amino acid or could be the substrates for aminoacylation. There are reasons to believe that tRNA not only preceded proteins, but may have preceded many other forms of RNA. Could an ancient, anteprotein-world function of these tRNA fragments be at play? And finally, is it possible that maternal and grandmaternal experiences are also handed down *via* an egg, as contrasted with a fetus coexperiencing the environmental insult? Investigators in the field of epigenetic intergenerational inheritance have focused on the paternal line for the obvious reason, the need to control for intrauterine events. But there are obvious experimental designs in animals where a female progenitor, most notably a grandmother, can be the experimental object, and never carry the fetus to be studied.

This newly uncovered tRNA fragment-mediated phenomenon is beguiling, and it may become one of the richest in all of modern biology, both satisfying our never-ending

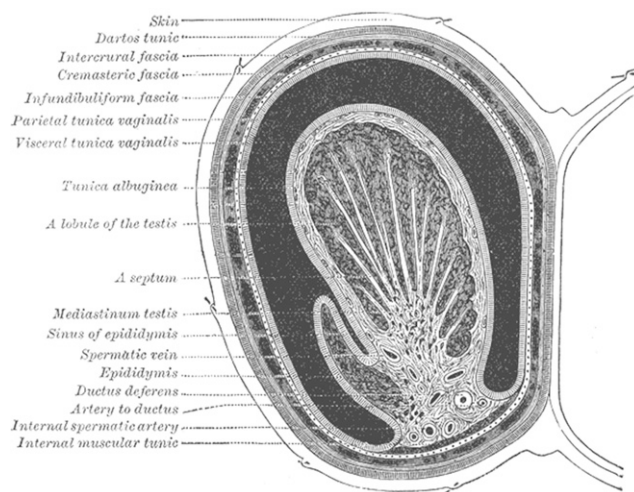


Figure 2. Human testis, Figure 1145 from *Anatomy of the Human Body*, Henry Gray, 1918.

curiosity and presenting obvious social dimensions we cannot ignore. FJ

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