Concluding Remarks

McFadden says that 'good science needs accurate communication' [1]. The merits of this statement can hardly be contested.

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Forum

Revisiting Trypanosome Mitochondrial Genome Mysteries: Broader and Deeper

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What do the products of a genome do, and when and why are they needed? For the protein products of the trypanosomatid parasites' mitochondrial genomes, the total expressed protein repertoire and the identities of the more difficult-to-characterize products have been challenging to acquire. Comparative genomics and new technologies may resolve that.

The Trypanosomatid Mitochondrial Genome

Trypanosomatids are protists that often parasitize the digestive tracts of insects. Studies exploring their basic biology have been richly rewarded. With their vast evolutionary distance from that of 'model' eukaryote species, unique means to execute proper gene expression and basic cellular functions have been discovered in trypanosomatids. For instance, the trypanosomatid single mitochondrion harbors a unique mitochondrial DNA structure with equally one-of-a-kind processes to facilitate maturation of its RNAs [1]. Typically its genome harbors two rRNA and 18 mRNA loci, but no tRNAs [2]. Protein products of mitochondrial genomes most often have roles within the organelle, most being subunits of the electron transport chain (ETC). Genomes may also contain proteins necessary for the generation or assembly of their encoded products. Most of the trypanosomatids' mitochondrion-encoded protein products have clear homologues in the mammalian system: at least eight NADH dehydrogenase (complex I) subunits (three actually encoded in the mammalian nucleus), a cytochrome bc_1 subunit, the three core cytochrome oxidase subunits, and one ATP synthase subunit. One product is a small subunit ribosomal protein [2].

Additional putative products of mitochondrial loci are called Maxicircle Unidentified Reading Frame 2 and 5 (MURF2, 5), and C (or G)-Rich 3 and 4 (CR3, 4); these very names reveal the difficulty in assigning a functional identity to these putative proteins [2]. CR3 and CR4 may be functional homologues of specific mammalian complex I subunits but do not share sequence similarity with the mammalian homologues [3,4]. ETC complexes exhibit subunit compositions that differ from the canonical eukaryotic systems [5], complex I in particular [3]. Nuclear- and mitochondrion-encoded ETC subunits must coordinate for functionality. Therefore, the sequences and expression of the subunits of mitochondrial origin cannot be ignored if we are to understand each ETC complex's biological roles and control. Investigation of the products of this mitochondrial genome has, however, proven challenging.

Current Challenges

For four reasons, the abundances, and sometimes the identities, of trypanosomatid mitochondrial genome products are uncharacterized [6]. The first is the aforementioned divergence of trypanosomatids from model eukaryotes that can make identification of homologues to some known proteins difficult, particularly those that are not core components of the ETC. Secondly, thus far the trypanosomatid mitochondrial genome has proven nonmanipulatable, so reverse genetics approaches are unavailable. Thirdly, the protein products of this genome have been notoriously difficult to detect by antibody detection or proteomics methods, although intact complexes containing these subunits can be observed. Finally, many mitochondrial mRNA loci encode cryptogenes. After these loci are transcribed, set numbers of uridylates (Us) are inserted and/or deleted in some or many positions within the mRNA. Once completed, this process of U-indel editing yields the translatable consensus mRNA [6]. Therefore, the number of functional mRNA loci in each species, or even the likely mature, translatable sequence of each cryptogene locus, cannot be elucidated from DNA alone (Figure 1).

U-indel editing begins at the most 3′ site requiring editing, and uses information

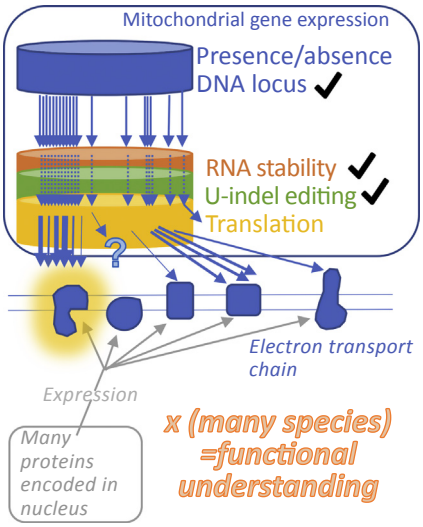

Figure 1. Determination of Whether a Species Functionally Edits a Cryptogene Transcript Requires Examination at the RNA Level. Checkmarks indicate features of the mitochondrial genome and its expression that can now be investigated in any trypanosomatid species from which genetic material can be obtained. The end goals are to determine the functions and regulatability of complexes and subunits of the electron transport chain originating from both the nucleus and mitochondria, and the function of any other protein products originating from the mitochondrial genome. Each blue arrow represents a mitochondrial mRNA locus, broken arrows represent expression, U-indel editing modification, and translation of the mitochondrial transcripts. Blue arrows of different thicknesses emerging from the rectangle represent the end products of mitochondrial gene expression, with variable line thicknesses indicating that products are present in different abundances. The lesser number of the blue arrows represent the fact that mitochondrial loci may not all be stably transcribed or converted to mature mRNA (conversely, alternative editing may result in more functional mRNAs than there are mitochondrial mRNA loci). The question mark indicates that we do not know the identities of all of the products of the genome. One electron transport chain complex is illuminated, representing complex I of the electron transport chain. Functional roles of this complex, or its subunits, in trypanosomatids will remain enigmatic unless both its nuclear- and mitochondrion-encoded subunits are examined.

contained on guide RNAs, also encoded in the mitochondrial genome, to edit a region of the transcript at multiple adjacent editing sites. Editing occurs in an overall 3′ to 5′ direction and can include insertion and deletion of hundreds of Us per transcript [6]. The large enzymatic and regulatory complexes controlling and coordinating editing are largely identified, though discrete roles for many proteins have yet to be elucidated [7]. U-indel editing and other mitochondrial molecular processes have been investigated within a small subset of trypanosomatids that are transmitted to a second host, particularly in those where the second host is a human or other socially or economically important mammal. In focusing on these species, the functional implications of post-transcriptional control in the mitochondrion, from stability to mRNA maturation, have been queried for a very tiny slice of trypanosomatid diversity [8]. This leaves two issues to address. One is the fact that what we consider to be the typical translatable mRNA output of the mitochondrial genome in trypanosomatids could be skewed by the subset of trypanosomes aggressively studied in the laboratory. The second is that we lack tools to explore the specific functions of the less-understood protein products of this genome. Fortunately, addressing the first problem may provide a tool to address the second problem.

Looking Forward

An approach to understanding control of trypanosomatid mitochondrial genome expression and the functions of its products is to compare them across strains and species. Trypanosomatid mitochondrial genomes are not identical in their complement of mRNA loci, although quite syntenic generally. For example, segments of the mitochondrial genome of the plant-parasitizing *Phytomonas* that contained mRNA loci have been deleted [9], in accordance with loss of cytochrome *c* reductase and oxidase activity, and loss of corresponding nuclear-encoded subunits. In order to determine the putative presence and sequence of cryptogene-derived edited mRNAs in different species, it is possible to align cryptogene DNA to previously determined edited mRNA of the best-studied trypansomatids *Trypanosoma brucei* and *Leishmania tarentolae*, synthetically inserting and/or deleting Us to achieve a likely edited, translatable product. However, a cryptogene transcript may not be ultimately edited even if it is theoretically possible (Box 1), possibly due to the disappearance of a necessary guide RNA. Studies reveal that sometimes U-indel editing is not very processive across a transcript [6]. If it is inefficient enough, no fully edited mRNAs will be produced, potentially yielding the same functional result as deletion of the genomic copy. In short, determining the complement of functional mitochondrial mRNAs generated in each species requires analysis at the RNA level.

Given the need for mitochondrial gene expression analysis in both one-host and two-host trypanosomatid species, it

Box 1. The Curious Case of U-indel Editing Loss in *Leishmania*

Leishmania is a genus of sandfly-transmitted trypanosomatids that infect mammals and reptiles. Using individual cloning and sequencing methodologies, a 1994 study revealed that a freshly-isolated *Leishmania tarentolae* strain completed editing of five specific cryptogenes, including loci for complex I subunits. This differed from a *L. tarentolae* strain in which editing initiated but did not progress to completion for these transcripts [12]. The editing-deficient strain was hypothesized to have lost the need for complex I (of ambiguous function in trypanosomatids) due to its long laboratory culture, losing guide RNAs essential to direct editing in some regions. However, a *L. mexicana amazonensis* strain that was also a product of long culturing retained full editing capacity of these same cryptogenes [4], even while other presumably virulent *Leishmania* species failed to retain full editing. Interestingly, the very existence of complex I has been called into question in *Leishmania*, at least in life-cycle stages that can be cultured. Therefore, it may be useful to revisit this variability in U-indel editing among *Leishmania* species with a wider representation of strains and species. Culture conditions may even be identified in which cryptogene-specific loss of editing is enhanced.

is fortuitous that high-throughput methods have arrived for identifying the sequences of edited mRNAs, and determining whether translatable products exist for each cryptogene [6]. New analytical methods to reconstruct the likely fully-edited canonical product from a subpopulation of individual reads take advantage of existing high-throughput RNA sequencing technologies. Once products are known, tools are available to explore the progression (or lack of progression) of U-indel editing of each transcript in more detail. High-throughput analyses are particularly important for another reason. The incidence of functional mRNAs resulting from noncanonical U insertions and deletions (i.e., 'alternative editing') remains ambiguous [6]. These may even encode something other than ETC subunits. Such mRNAs are best detected by unbiased deep-sequencing methods. Messenger RNA high-throughput sequencing, combined with guide RNA population information (e.g., [10]) can be used in comparative studies to learn what products are made, and what their relative abundances are, under what circumstances. Of course, mass spectrometry methodologies are also improving apace, and we must additionally take every opportunity to apply these to the analysis of mitochondrion-encoded proteins.

A major argument in favor of multispecies analyses of mitochondrial genome expression is that differences in the composition and functionality of the trypanosomatid ETC are known to exist among species. One study specifically surveyed mitochondrial respiration in one-host species and found a plethora of differences that were, interestingly, unrelated to apparent phylogeny [11]. The origin of many of those differences will be the nuclear genome and its expression, but some may be of mitochondrial origin. Furthermore, the unidentified mitochondrial genes of the trypanosomatids are also likely to be part of, or influence, the ETC, and they are found in most trypanosomatid genomes. Finally, ongoing investigations into various possible biological roles of complex I, seemingly different among species, would benefit from a broader analysis [3] (Box 1). Ultimately, in characterizing products of the mitochondrial genome in diverse trypanosomatids, we will attain a more representative view. The explosion of newly sequenced genomes and available U-indel transcriptome sequencing analysis tools argues that the time for this approach is now.

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Forum

Advancing the Development of a Human Schistosomiasis Vaccine

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Three vaccines against human schistosomiasis are in different phases of clinical development, and a fourth is expected to enter the clinic soon. Successful introduction of an efficacious preventive human schistosomiasis vaccine will require integration into existing health systems such as those that deliver childhood vaccines or mass drug administration programs.

Schistosomiasis has one of the highest disease burdens of the neglected tropical diseases (NTDs), especially in Africa, where more than 90% of infections occur. The Global Burden of Disease Study 2016 estimates that 190 million people are infected with schistosomes, with more than 70 million new infections and thousands of deaths occurring annually [1]. Alternative estimates suggest that the prevalence and mortality attributable to this NTD may be much higher, with an estimated 300 000 people dying each year in Africa alone due to the chronic sequelae of infection [2]. In addition to the morbidity and mortality directly associated with schistosome infection, accumulating evidence points to the role of urogenital schistosomiasis caused by *Schistosoma haematobium* in promoting susceptibility to HIV in