Rho, Rho, Rho Your Cells

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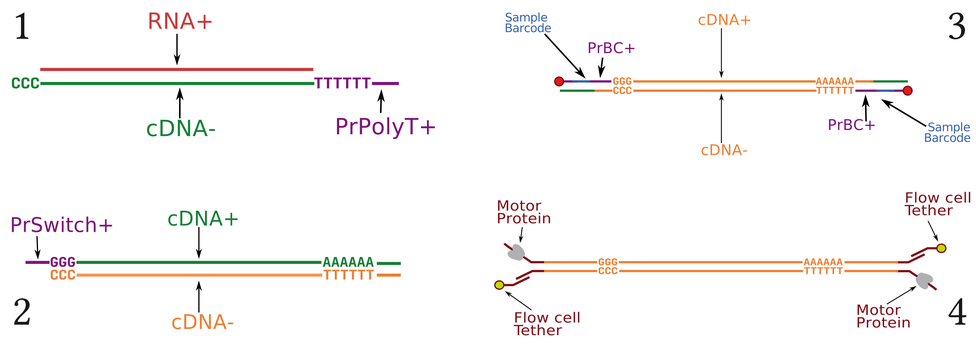
Introduction

Using the mouse 4T1 breast carcinoma cell line, we have investigated whether nuclear expression of mitochondrial pseudogenes might contribute to an apparent uneven pattern of mitochondrial expression by removing mitochondrial DNA from cells and carrying out strand-switch cDNA sequencing with the MinION. The current understanding is that the entire mitochondrial genome is transcribed in both directions, and these multi-gene transcripts are processed into individual genes, 13 of which are translated into proteins, and three long non-coding RNAs.

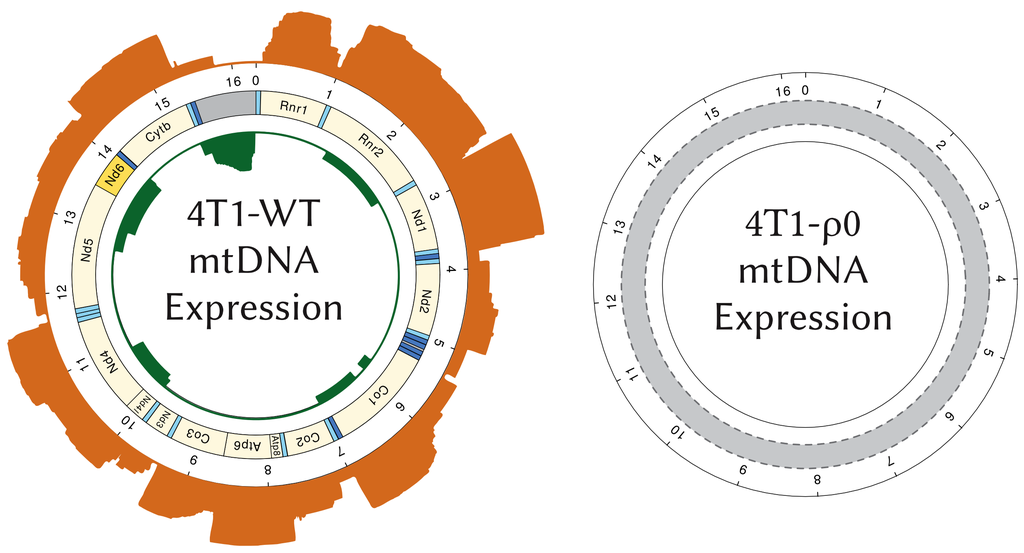
Strand-switch cDNA Prep

1. cDNA extended from polyT to the start of the gene
2. Forward strand extended from strand-switch anchor
3. PCR attaches barcodes and adapter anchor to cDNA
4. ONT adapters attached prior to flow-cell loading



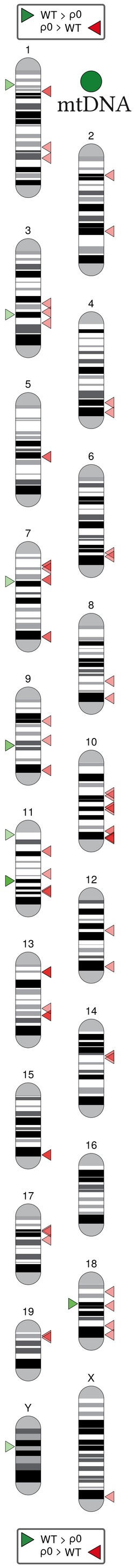
Strand-specific Mapping

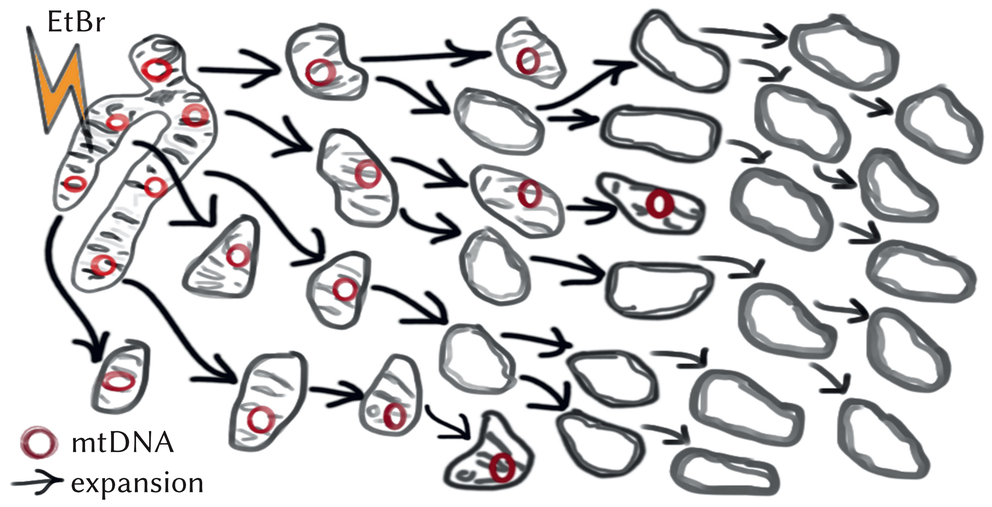
The primer and barcode sequences are extended from the cDNA in a specific orientation. Using this knowledge, it is possible to determine the direction of transcription from nanopore cDNA reads (see below).



Loss of mtDNA Expression

The genes in the mitochondrial genome are mostly (but not entirely) encoded on the same strand of mitochondrial DNA. Expression of mitochondrial genes is completely lost in the 4T1-ρ0 cell line, indicating that there is no nuclear expression of mitochondrial pseudogenes.





Generating Rhos

The most effective way to remove mitochondrial DNA from cells is to stop them from producing it in the first place. Cells are cultured with low-dose ethidium bromide for a few months in a media containing pyruvate and uridine. Mitochondria in the resulting 4T1-ρ0 cell line are missing their characteristic stacked cristae, and lose almost all their respiratory function.

Nuclear Gene Expression

Chr	Loc (Mb)	Gene	4T1-WT	4T1-ρ0
11	82.0	*Ccl2*	316	0
1	24.6	*[Col19a1]*	246	0
18	32.8	*Tslp*	69	6
7	43.8	*Klk10*	4	154
15	101.4	*Krt7*	0	264
13	86.7	*[Cox7c]*	0	365

Throughout most of the nuclear genome, the mapped coverage of cDNA is similar between 4T1-WT cells and 4T1-ρ0 cells without mitochondrial DNA, but not everywhere. Six regions are shown above that demonstrate large read counts differences, with reads primarily in 4T1-WT (above) and 4T1-ρ0 (below). Closest gene names in *[italic]* indicate that the region of difference is outside the gene. In most locations, the 4T1-ρ0 cells had increased coverage (see centre figure).

Concluding Remarks

Removing mitochondrial DNA prevents the expression of mitochondrial genes. An additional exploration of nuclear genes unearthed a number that had increased coverage in 4T1-ρ0 cells, suggesting that loss of mitochondrial DNA alters the transcription of nuclear genes. This preliminary discovery will be extended by looking in more depth at transcriptional differences between other wild-type and ρ0 cell lines.

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