Nuclear Transcript Expression is Changed in the Absence of mtDNA and Respiration

David Eccles[1], Carole Grasso[1], Stepana Boukalova[2], and Mike Berridge[1]
1 Malaghan Institute of Medical Research, Wellington, New Zealand
2 Institute of Biotechnology, Czech Academy of Sciences, 252 50, Vestec, Prague-West, Czech Republic

Introduction

We investigated changes in nuclear transcript expression in mouse 4T1 breast carcinoma cell lines after depletion of mitochondrial (mt) DNA (**4T1-ρ⁰**), and whether expression was recovered following mitochondrial acquisition and respiration recovery in sub-cutaneous (**ρ⁰ SC**) and metastatic lung tumours (**ρ⁰ SCL**).
Four different cell lines were multiplexed onto MinION flow cells and sequenced using the cDNA kit, then mapped to the genome and transcriptome.

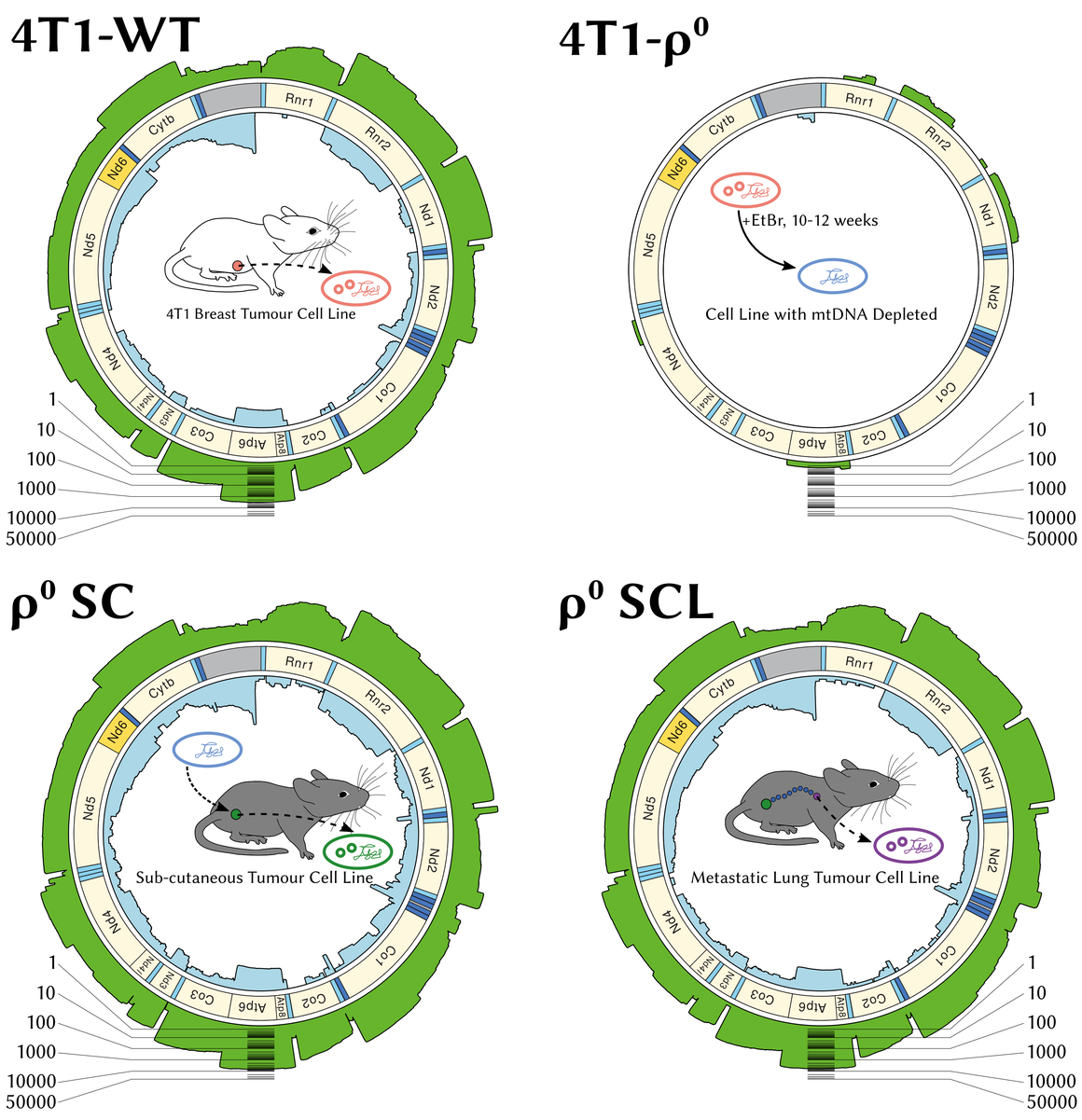

Fig 1: mtDNA Expression in mouse tumour cell lines (FWD/REV). The transcript coverage is represented radially on a log scale to accomodate substantial expression differences for different genes within the mitochondrial genome. Most mitochondrial gene transcripts are encoded on the forward strand, with less expression on the reverse strand. Mitochondrial expression is almost completely absent in **the 4T1 ρ⁰** cell line.

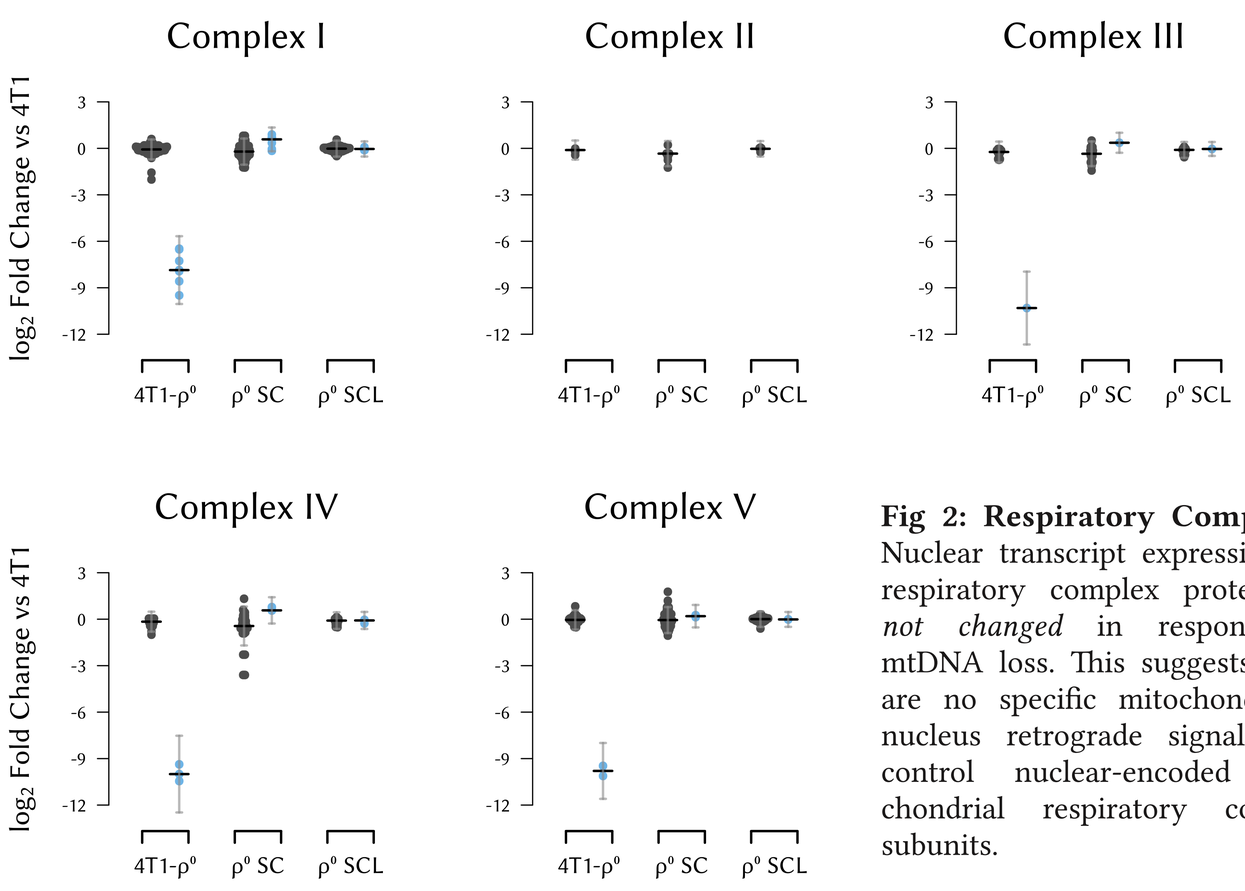

Fig 2: Respiratory Complexes. Nuclear transcript expression for respiratory complex proteins *is not changed* in response to mtDNA loss. This suggests there are no specific mitochondria-to-nucleus retrograde signals that control nuclear-encoded mitochondrial respiratory complex subunits.

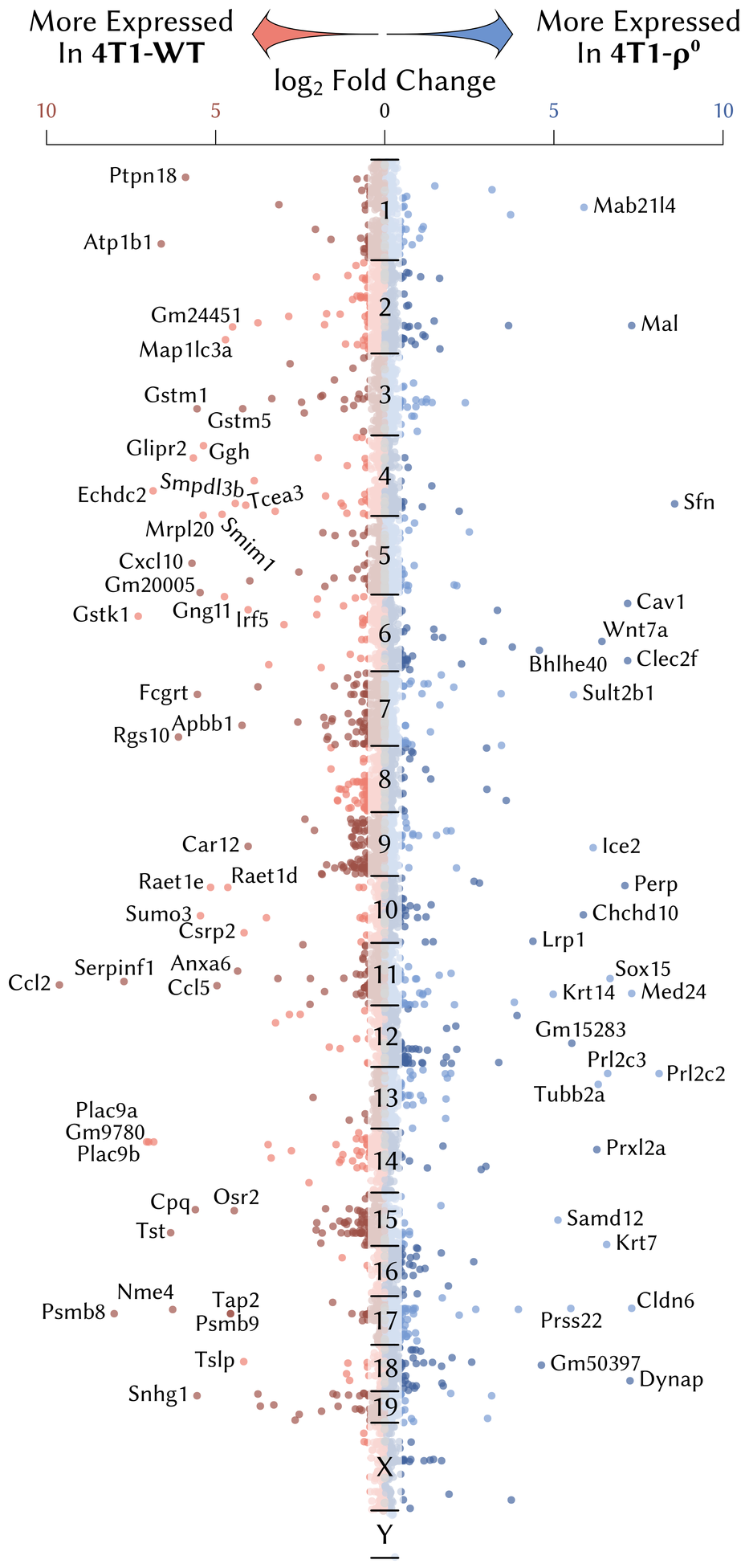

Fig 3: Genomic location of differentially-expressed 4T1-WT and ρ⁰ transcripts. Differentially-expressed transcripts are distributed throughout the entire genome, with similar numbers of transcripts up and down regulated in response to the loss of mitochondrial DNA and therefore respiratory function. This suggests a systemic change in cellular function.

Summary

Loss of mtDNA is an extreme cellular phenotype; lack of change in nuclear respiratory complex transcripts following mtDNA depletion suggests the cell does not have inbuilt mechanisms to explicitly detect the loss of respiratory complexes. However, gene transcript expression is altered in over 200 genes following loss of mitochondrial DNA (with subtantial activation or suppression in 68 genes), suggesting that the loss of respiratory function triggers extensive changes in cellular activity.

Funding: Health Research Council, the Cancer Society of New Zealand, and the Malaghan Institute of Medical Research