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Interactions between mitochondrial and nuclear DNA in mammalian cells are non-random

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ABSTRACT

Chromosome Conformation Capture techniques regularly detect physical interactions between mitochondrial and nuclear DNA (*i.e.* mito-nDNA interactions) in mammalian cells. We have evaluated mito-nDNA interactions captured by HiC and Circular Chromosome Conformation Capture (4C). We show that these mito-nDNA interactions are statistically significant and shared between biological and technical replicates. The most frequent interactions occur with repetitive DNA sequences, including centromeres in human cell lines and the 18S rDNA in mouse cortical astrocytes. Our results demonstrate a degree of selective regulation in the identity of the interacting mitochondrial partners confirming that mito-nDNA interactions in mammalian cells are not random.

1. Introduction

It is widely understood that different mammalian cell types maintain diverse numbers of mitochondria due to differences in their energetic requirements. This is attributed to the central role that mitochondria play in cell metabolism and energy production *via* oxidative phosphorylation. However, mitochondria are also known to be involved in cellular processes that are not directly related to metabolic energy generation including cellular differentiation, the control of cell growth and cell death (Duchen, 2004).

Mitochondria contain their own genomes. Unlike the metazoan nuclear genome, the mitochondrial genome is only ~16 kb (human and mouse) and is present in multiple copies within the mitochondrial organelles in each cell. Moreover, the ratio of mitochondrial:nuclear genomes depends on the cell type (Clay Montier et al., 2009). Mitochondrial and nuclear functions are highly interdependent in a reciprocal manner (Horan et al., 2013). This is reflected in the fact that while the mitochondrial encoded genes are essential for mitochondrial function, >1000 proteins required for mitochondrial function are encoded in the nucleus (Andersson et al., 2003). Therefore, the assembly of functional mitochondria requires coordinated expression of genes within both the nuclear and mitochondrial organelles. Consequently, a mechanism to co-ordinate and control the expression of the nuclear and mitochondrial encoded genes must exist (Butow and Avadhani, 2004; Poyton and McEwen, 1996) to facilitate constant communication between mitochondria and nuclei.

The mitochondrial genome has dramatically reduced in size over the course of its evolution as an endosymbiont. In fact, functional transfer of the majority of (~98%) of mitochondrial genes required for mitochondrial functions into the nucleus has occurred over the past 1.5 billion years (Bock and Timmis, 2008; Timmis et al., 2004). The ongoing nature of this transfer is reflected in the finding that the nuclear chromosomes of a wide range of eukaryotic species contain nuclear mitochondrial sequences (NUMTs) that are homologous to contemporary mitochondrial DNA (mtDNA) sequences (Hazkani-Covo et al., 2010; Ricchetti et al., 2004). Transfer of mtDNA into the nucleus and nuclear chromosomes has been measured in germ-line and somatic cells in many mammals, with rates between 5.1–5.6 $\times$ 10^{-6} per cell/per generation in the germ-line (Ricchetti et al., 2004; Bensasson et al., 2003; Ju et al., 2015) and 2 $\times$ 10^{-4}per cell/per generation in somatic cells (Ju et al., 2015). The transfer of mtDNA in somatic cells is not just intracellular and recent work has described instances where mtDNA and intact functional mitochondria are transferred between cells (Liu et al., 2014; Tan et al., 2015;

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Falchi et al., 2012; Spees et al., 2006; Ahmad et al., 2014). Therefore, it remains possible that both intracellular and intercellular mtDNA transfer is involved in biological functions that are not yet fully described.

De novo mtDNA insertions into the genome in germ-line cells are established as resulting in either neutral or harmful polymorphisms (Hazkani-Covo et al., 2010). However, the genetic, functional and phenotypic outcomes associated with mtDNA insertions into the nuclear chromosomes of somatic cells are less defined. Despite this, mtDNA transfer rates into nuclear chromosomes are known to be dynamic and alter with differentiation (Schneider et al., 2014), age (Caro et al., 2010) or cancer progression (Ju et al., 2015).

There are several mechanisms that could facilitate the transfer of mtDNA into the nucleus in somatic cells despite the presence of many physical barriers that have to be overcome. Physical exchange of mitochondrial components (including mtDNA) could be promoted by physical associations between the mitochondrial and nuclear membranes (Mota, 1963). Alternatively, vacuole-mediated pathways could carry mtDNA into the nucleus during mitochondrial turnover, as in the case with the yeast nuclear mitochondrial escape (*yme*) mutants where disruption of the vacuole mediated pathway is hypothesized to enable elevated transfer of mtDNA into the yeast nucleus (Campbell and Thorsness, 1998; Hanekamp et al., 2002; Park et al., 2006; Shafer et al., 1999). Moreover, during mitochondrial fusion, fission (Dimmer and Scorrano, 2006), or degradation (Ding and Yin, 2012) mitochondrial genome fragments or entire genomes can be released into the cell cytoplasm before subsequently being engulfed by the cell nucleus (Shafer et al., 1999). Finally, mtDNA could be transferred into the nucleus as mRNA or cDNA (complementary DNA) (Nugent and Palmer, 1991; Rodley et al., 2012) relying upon a reverse transcriptase within the mitochondria, cytoplasm or nucleus to complete the process.

The formation of NUMTs requires that mtDNA associate directly with the chromosomes. Therefore, once the mtDNA has transferred into the nucleus, and before it is inserted into the nuclear chromosomes, it must interact with the nuclear DNA (mito-nDNA interactions). Genome Conformation Capture (GCC) has been used previously to capture specific mito-nDNA interactions occurring within nuclei of the budding (Rodley et al., 2009) and fission yeasts (Grand et al., 2004). Collectively, the formation of the mito-nDNA interactions appears to be dynamic and dependent upon the energetic state in budding yeast (Rodley et al., 2009; Rodley et al., 2012) and cell cycle stage in fission yeast (Grand et al., 2004). Finally, the mito-nDNA interactions in budding yeast are functional (Cheng and Ivessa, 2010; Spees et al., 2006) and associated with the regulation of the interacting nuclear gene's transcript levels (Spees et al., 2006; Cheng and Ivessa, 2010; Rodley et al., 2012).

High-resolution Chromosome Conformation Capture (HiC) techniques regularly capture mito-nDNA interactions within mammalian nuclei (Dixon et al., 2012; Rao et al., 2014). However, the mito-nDNA interactions that are captured by HiC are routinely considered as random artefacts. The central argument for the classification of mito-nDNA interactions as being random centres on the fact that mtDNA and nuclear chromosomes reside in different organelles. As such, it is assumed to be highly unlikely that mtDNA migrates through multiple membranes to interact specifically with nuclear DNA (Dixon et al., 2012). Rather, mito-nDNA interactions are assumed to form as a result of chromatin diffusion, random collisions and ligation between the numerous mtDNA and nuclear restriction fragments during the ligation step of the HiC protocol. However, comprehensive statistical evaluation of the randomness of mito-nDNA interactions captured by HiC experiments has been lacking and it has been assumed that the observed differences in mito-nDNA interactions captured by the *in situ* and diluted HiC preparations confirm the randomness of these interactions (Rao et al., 2014).

Here, we have evaluated mtDNA interactions captured: 1) by HiC in six human cell lines; and 2) by 4C in mouse cortical astrocytes. We show that mito-nDNA interactions are statistically significant and shared between multiple replicates.

2. Methods

2.1. Mining HiC data for mitochondrial-nuclear interactions

The mapping locations of HiC read pairs, minus low quality alignments and duplicates, were obtained from Rao et al. GSE63525 (Rao et al., 2014) for six human cell lines (GM12878, IMR90, K562, KBM7, NHEK and HUVEC; Supplementary Table S1). HiC libraries for different biological and technical replicates were chosen for analysis if their sequencing depths were $1.07 \times 10^8 < \times < 3.9 \times 10^8$ and varied by $\pm 43\%$ (Supplementary Spreadsheets; S1). HiC read pairs that mapped simultaneously to the mitochondrial and nuclear chromosomes were selected, filtered for alignment quality (MAPQ ≥ 30). 50 bp was added to left coordinate of the mitochondrial and nuclear partners provided within the files of HiC read pairs to obtain a 3′ coordinate for the interacting partners. The proximity of the mapped sequences to MboI (^GATC) restriction sites was checked. Specifically, fragments had to map within 450 bp of an MboI restriction site, which is less than the maximum insert size (500 bp), in order to be considered real. The coordinates for interactions filtered for proximity to the nuclear and mitochondrial MboI restriction sites interacting partners were deposited in bed format (Supplementary information).

The fasta sequences of the nuclear loci captured interacting with the mtDNA were obtained from human genome (hg19) using fastaFromBed – BEDTools (Quinlan and Hall, 2010) and mapped onto (Homo_sapiens GRCh37.75 ensemble release) using bowtie 2 (version 2.1.0) with the following parameters "-k 1 -D 20 -R 3 -N 0 -L 20 -i S,1,0.50 -p 8" (Langmead and Salzberg, 2012). Mapping was performed to identify for repetitive sequences. No mismatches were permissible during the mapping onto the reference genome (Supplementary information).

The number of nuclear loci in each replicate and the number of nuclear loci shared between pairs of all replicates for a cell line was then counted at the MboI restriction fragment level (Supplementary Spreadsheets; S2). Nuclear loci that overlapped known human NUMTs (Calabrese et al., 2012) (Supplementary Spreadsheets; S3) and those mapping to chrY were excluded from the analysis due to the repetitive nature of these sequences and inaccuracy associated with mapping to these regions.

We used the Fisher's exact test to determine the statistical significance of the observed overlap for nuclear partners that were captured interacting with the mtDNA in pairs of replicates. The parameters for this analysis were: K - number loci found to interact with mtDNA in the first replicate of the pair; n - number loci found to interact with mtDNA in the second replicate of the pair; k – number of shared nuclear loci between the two replicates; N – number of all possible MboI restriction fragments in the genome (excluding those within the mitochondrial genome, chrY and overlapping NUMTs coordinates) (Supplementary Table S3). The statistical test was performed using R (R Core Team, 2013).

2.2. Gene ontology (GO) term enrichment analysis of the HiC data

Genes that overlapped or flanked the nuclear loci that were captured interacting with the mtDNA, in all replicates of a cell line, were identified. Default parameters within the AmiGO Term Enrichment online tool (http://amigo.geneontology.org/amigo) were used to determine if the captured nuclear loci were enriched in particular GO functional groups.

2.3. Estimating the distribution of interactions on the human mtDNA

Samtools (Li et al., 2009) was used to obtain the nuclear interacting fragments that corresponded to the captured nuclear loci, filtered for proximity to MboI restriction site, that were shared between all the replicates of each cell line from the sam files (Supplementary information). The corresponding mitochondrial partners were extracted from the bed