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Mitochondrial disease activates transcripts of the unfolded protein response and cell cycle and inhibits vesicular secretion and oligodendrocyte-specific transcripts

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Abstract

Mutations in gene products expressed in the mitochondrion cause a nuclear transcriptional response that leads to neurological disease. To examine the extent to which the transcriptional profile was shared among 5 mitochondrial diseases (LHON, FRDA, MELAS, KSS, and NARP), we microarrayed mutant and control groups in N-tera2, SH-SY5Y, lymphoblasts, fibroblasts, myoblasts, muscle, and osteosarcoma cybrids. Many more transcripts were observed to be significantly altered and shared among these 5 mitochondrial diseases and cell types than expected on the basis of random chance, and these genes are significantly clustered with respect to biochemical pathways. Mitochondrial disease *activated* multiple transcripts of the unfolded protein response (UPR), and of the cell cycle pathway, and low doses of the mitochondrial inhibitor rotenone induced UPR transcripts in the absence of cell death. By contrast, functional clusters *inhibited* by mitochondrial disease included: vesicular secretion, protein synthesis, and oligodendrogenesis. As it is known that UPR activation specifically inhibits vesicular secretion and protein synthesis, these data support the view that mitochondrial disease and dysfunction triggers the UPR, which in turn causes secretory defects which inhibit cellular migratory, synaptic, and oligodendrocytic functions, providing a testable hypothesis for how mitochondrial dysfunction causes disease. Since ischemic hypoxia, chemical hypoxia, and mitochondrial genetic disease (which could be considered 'genetic hypoxia') produce an overlapping induction of UPR and cell cycle genes which appears to have negative consequences, the modulation of these responses might be of benefit to patients with mitochondrial disease.

Keywords: Mitochondrial genetic disease; Microarray; Unfolded protein response

1. Introduction

Pathogenic mutations in gene products expressed in the mitochondrion, whether they are originally encoded in the mitochondrion or the nucleus, cause mitochondrial disease. Patients with mitochondrial disease present prominently with neurological signs include ataxia, myoclonus, dementia, optic neurodegeneration, retinitis pigmentosa, muscle weakness/paralysis, deafness, and also diabetes (DiMauro, 2004); some of these signs occur in individual mitochondrial diseases (for example ataxia in Friedreich's ataxia), and some are shared among mitochondrial diseases (for example optic neurodegeneration in LHON and Friedreich's ataxia).

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The nucleus must produce a transcriptional response to each mitochondrial disease mutation, which must at some level determine the pathophysiological consequences of the mutation. To address to what extent the nuclear response is shared among different mitochondrial diseases, we have carried out microarray analysis on at least 2 representative cell types of five mitochondrial diseases: Leber's Hereditary Optic Neuropathy (LHON), Friedreich's ataxia (FRDA), Neurogenic Ataxia and Retinitis Pigmentosa (NARP), Myoclonic Epilepsy with Ragged Red Fibers (MERRF), and Kearns–Sayre Syndrome (KSS); and also on an osteosarcoma cell line with a complete depletion of mtDNA, a rho-zero cell line, as a model of mtDNA depletion diseases. A total of 9 different cell types with mitochondrial defects were compared, although not every mutation was available in every cell type. The complete data set includes 22 groups of comparisons of mutants versus controls. Two microarray studies comparing parental and rho-zero (lacking mitochondrial DNA) cells have been previously performed (Behan et al., 2005; Delsite et al., 2002).

We observed that there was much more sharing of transcriptional profile among mitochondrial diseases than expected on the basis of random chance, i.e. if the nuclear response to each mitochondrial disease and in each cell type were completely independent. The shared elements of the mutant profiles emphasize particular transcriptional and biochemical pathways, from which we infer some shared transcriptional (and pathological) consequences of mitochondrial disease. Interestingly, some of these pathways are also shared with ischemic hypoxia and chemical hypoxia, suggesting that the nucleus responds in a common way to mitochondrial dysfunction, whether the mitochondrial defect is the result of genetic mutations, oxygen depletion, lack of membrane potential, or decreased electron flow.

2. Materials and methods

2.1. Cell lines and culture conditions

In total 9 cell types with mitochondrial disease were compared, these include: lymphoblasts, osteosarcomas, fibroblasts, myoblasts, differentiated muscle, NT2 neural cells undifferentiated and differentiated, and SH-SY5Y neuroblastoma cells undifferentiated and differentiated.

2.2. Fibroblasts

Seven control fibroblast cell lines were obtained from Coriell (GM00024, GM00321, GM01653, GM01863, GM03440, GM08402, and GM13335). Four fibroblast cell lines derived from patients with Friedreich's ataxia (p13, p1037, p1143, and p3) have been previously described (Wong HMG 1999, Tan HMG 2003). Two fibroblast cell lines derived from KSS patients were acquired from both the University of Miami Brain and Tissue Bank (Miami, Fl) (3246 and 3511) and from Dr. Nancy Kennaway (AC and RJ). LHON fibroblast cell lines were derived from seven individual patients with LHON, 1 bearing the 14484 mtDNA mutation (L180 from A. Martinuzzi) and 6 bearing the 11778 mutation (HFBS and HFGJ were kind gifts from E. Holme Goteborg University, Sweden, HFDG and HFFF from A. Martinuzzi, EICbIII and EIJbIII were kind gifts from S. Marzuki, Eijkman Institute for Molecular Biology, Indonesia) (Vergani et al., 1995; Sudoyo et al., 2002). NARP fibroblast cell lines were obtained from two separate patients (602 was a kind gift from M. Zeviani, National Neurological Institute Carlo Besta, Milan, Italy, and 1075 was from F. Taroni). All fibroblast cell lines were grown as previously described (Wong et al., 1999).

2.3. Lymphoblasts

Control lymphoblast cell lines (GM00333, GM00536 and GM00621) were obtained from Coriell cell repositories (Camden, NJ). Lymphoblasts from three patients with FRDA (p131, p218, and p585) have been previously described (Tan et al., 2001). Lymphoblast cell lines 848 (a kind gift from S. Zullo, NIH Bethesda, MD) and 63358 (a kind gift from G. Matthijs) (Tabaku, 1999) contained the common 4977 mtDNA deletion and a 7.4 kB mtDNA deletion, respectively. Lymphoblast cell lines bearing LHON mutations derived from four different patients (980002, 980004 bearing the 11778 mtDNA mutation and 11605, 910615 bearing the 3460 mtDNA mutation) were detailed previously (Danielson et al., 2005). Two lymphoblast cell lines bearing MERRF mtDNA mutations (GM11907 and GM11906) were obtained from Coriell. The lymphoblast cell lines bearing NARP mtDNA mutations (GM13740 and GM13741) were obtained from Coriell. All lymphoblast cell lines were maintained as described in Tan et al. (2001).

2.4. Undifferentiated NT2

An NT2 FRDA cell model was generated by using RNA interference to deplete frataxin levels (NT2FRTXN-), control NT2 cells were transfected with an empty vector as previously described in Tan et al. to use as controls for the frataxin-deficient NT2 cells (Tan et al., 2003). All other NT2 controls (CM and CS) used were transmitochondrial cybrids generated as described previously (King and Attardi, 1989). Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation using mtDNA which contains no mutations associated with any human disease (King and Attardi, 1989). Two NT2 transmitochondrial cybrid cell lines (4.9-1 and 4.9-2) bearing the common 4977 deletion were generated as described previously (King and Attardi, 1989; Wong et al., 2002). Three transmitochondrial cybrid NT2 cell lines (MD, ME, and MF) were generated which bear the 11778 LHON mtDNA mutation using the methods described in Wong et al. (2002). All NT2 cells were maintained as described in Wong et al. (2002).

2.5. Differentiated NT2

Three NT2 cell lines were generated (11778-1, 11778-2 and 3460-1) which contain the 11778 and 3460 mtDNA mutations and a control NT2 (BNT2) cell line were differentiated into neural cells as documented previously (Wong et al., 2002) before microarray analysis was conducted.

2.6. SHSY5Y

Three control (CD, CE and CG) and three LHON (MA, MB, and MC all containing the 11778 mtDNA mutation) SHSY5Y transmitochondrial cybrid cell lines were generated as previously described (King and Attardi, 1989). SHSY5Y cells were maintained in the same media as osteosarcoma (see below).

2.7. Differentiated SHSY5Y

The control (CE) and LHON (MA and MC) SHSY5Y cell lines were differentiated into neural cells as described in Wong et al. (2002).

2.8. Osteosarcoma

The three control osteosarcoma 143B transmitochondrial cybrid cell lines have been previously documented (HGA13, HPC7, and H1959) all contain no mtDNA mutations associated with human disease (Danielson et al., 2002). Four 143B transmitochondrial cybrids bearing mtDNA deletions which cause KSS were used 51-18, 14-6 (contain the common 4977 mtDNA deletion were kind gifts from Y.H. Wei (Pang et al., 1999), 3.7 and 16.3 (contain a 7.5 kB mtDNA deletion were kind gifts from C. Moraes) (Sancho et al., 1992). Five 143B cybrid cell lines bearing LHON mutations were utilized in this study, HCT1, HFF3, HPE9 (all containing the 11778 mtDNA mutation) RJ206, and HMM5 (both containing the 3460 mtDNA mutation) (Danielson et al., 2002). Two 143B cybrid cell lines bearing the mtDNA mutation at nucleotide 8344 associated with MERRF were used, R11A (a kind gift from G. Attardi and A. Chomyn, Cal Tech, CA) and MERRF (a kind gift from E. Shoubridge, McGill University, Canada). Additionally two 143B cybrids designated 261 and 239 were used (kind gifts from G. Manfredi, Cornell University, NY) which contained the mtDNA mutation at nucleotide position 8993 associated with NARP. The 143B osteosarcoma rho-zero cell line was a kind gift from M. Zeviani. All osteosarcoma cell lines were maintained as described in Danielson et al. (Danielson et al., 2002).

2.9. Muscle

Seven snapfrozen skeletal muscle samples from patients diagnosed with KSS (1320, 1669, 2576, 1348, 3515, 3516, and 3517) along with skeletal muscle samples from four healthy patients (2378, 2666, 2794, and 2815) were utilized (all muscle samples were kind gifts from S. DiMauro, Columbia University, NY).

2.10. Myoblast

Two control (2FO135 and 2FO873 from Cambrex San Diego, CA) and two KSS myoblast cell lines (8279 and 9317 both kind gifts from F. Taroni) were utilized. Myoblasts were cultured in skeletal muscle basal media (SkBM) supplemented with hEGF, 10% fetal bovine serum, and L-glutamine in a humidified incubator containing 5% carbon dioxide at 37 °C. All cells grew normally and were mycoplasma free. All cell lines were stored in liquid nitrogen until needed for experiments, at which time they were revived, and grown for a limited number of passages (~10) to isolate RNA for microarray analysis.

2.11. Microarray analysis

We utilized Affymetrix U95Av2 oligonucleotide chips which represent 12,599 human transcripts (9091 unique human genes) to identify differentially expressed genes. In the Affymetrix format, transcripts are often represented by multiple probe sets. Microarray samples were made as per Affymetrix protocol (Santa Clara, CA) and as described in Danielson et al. (Danielson et al., 2005). Microarray data files were analyzed using DNA-Chip Analyzer (dChip) software (Li and Wong, 2001). Differentially expressed genes were identified by comparing GeneChips (within the same cell type) designated as baseline (e.g. unfused control cell lines) to GeneChips that represent the experimental parameter (e.g. Disease such as LHON) using the following criteria, a difference in mean fluorescence intensity between baseline and experimental $\geqslant 50$, and a p value < 0.05. For each GeneChip, biotin labeled cRNA from one cell line was hybridized according to manufacturer's (Affymetrix) specifications. The cell lines used for each microarray comparison are illustrated in Table 1, the number in parentheses indicating the number of replicates done for each cell line. Gene profile analysis was then done to determine if there are any relationships between the genes with altered expression based on their subcellular localization or biological process using the program OntoExpress (Khatri et al., 2002).

2.12. QRT-PCR

Total RNA was prepared from cells using an RNeasy minikit (Qiagen). Superscript reverse transcriptase II (Invitrogen, Carlsbad, CA) was used to create cDNA starting with 1 μg of total RNA. The following primers were used for amplification: COUP-TFII forward 5′-GACAAG CAGCAGCAGCAGC-3′ and reverse 5′-CGAACTGCCC GTGGGTCGG-3′; Glycoprotein M6A forward 5′-ATTT TGCTGATGGTGGAAGG-3′ and reverse 5′-CTCCAGC AAGTGCCACAATA-3′; CHOP forward 5′-ATTCCAG

Table 1
Microarray comparisons done

Disease	Cell type	Control	Mutant	Genes with altered expression
FRDA	Fibroblast	GM00024 (2) GM00321 (2) GM01653 (1) GM01863 (2) GM03440 (1) GM08402 (1) GM13335 (1)	p13 (1) p1037 (1) p1143 (1) p3 (1)	531
FRDA	Lymphoblast	GM00333 (3) GM00536 (3) GM00621 (3)	p131 (2) p218 (2) p585 (2)	1259
FRDA	NT2	NT2Ctl.mock (6)	NT2FRTXN- (5)	143
KSS	Fibroblast	GM00024 (2) GM00321 (2) GM01653 (1) GM01863 (2) GM03440 (1) GM08402 (1) GM13335 (1)	3246 (1) 3511 (1) AC (1) RG (1)	643
KSS	Lymphoblast	GM00333 (3) GM00536 (3) GM00621 (3)	848 (1) 63358 (1)	671
KSS	NT2	CM (2) CS (2)	4.9-1 (1) 4.9-2 (1)	1876
KSS	Osteosarcoma	HGA13 (3) HPC7 (1) H1959 (1)	51-18 (2) 14-6 (2) 16.3 (2) 3.7 (2)	1312
KSS	Muscle	2378 (1) 2666 (1) 2794 (1) 2815 (1)	1320 (1) 1669 (1) 2576 (1) 1348 (1) 3515 (1) 3516 (1) 3517 (1)	30
KSS	Myoblast	2FO135 (1) 2FO873 (1)	4317 (1) 8279 (1)	377
LHON	Fibroblast	GM00024 (2) GM00321 (2) GM01653 (1) GM01863 (2) GM03440 (1) GM08400 (1) GM08402 (1) GM13335 (1)	L180 (2) HFBS (2) HFDG (2) EICB III (2) EICJ III (1) HFFF (1) HFGJ (2)	273
LHON	Lymphoblast	GM00333 (3) GM00536 (3) GM00621 (3)	11605 (1) 910615 (1) 980002 (1) 980004 (1)	526
LHON	Osteosarcoma	HGA13 (3) HPC7 (1) H1959 (1)	HCT1 (3) HFF3 (3) HPE9 (1) RJ206 (3) HMM5 (3)	1092
LHON	NT2	CM (2) CS (2)	MD (2) ME (2) MF (2)	325

Table 1 (*continued*)

Disease	Cell type	Control	Mutant	Genes with altered expression
LHON	SHSY5Y	CD (2) CE (2) CG (2)	MA (2) MB (2) MC (2)	525
LHON	Differentiated NT2	BNT2 (2)	NT2-11778-1(2) NT2-11778-2 (3) NT2-3460-3 (2)	395
LHON	Differentiated SHSY5Y	CE (2)	MA (2) MC (2)	387
MERRF	Osteosarcoma	HGA13 (3) HPC7 (1) H1959 (1)	R11A (1) MERRF (1)	598
MERRF	Lymphoblast	GM00333 (3) GM00536 (3) GM00621 (3)	11907 (1) 11906 (1)	256
NARP	Fibroblast	GM00024 (2) GM00321 (2) GM01653 (1) GM01863 (2) GM03440 (1) GM08402 (1) GM13335 (1)	602 (1) 1075 (1)	345
NARP	Lymphoblast	GM00333 (3) GM00536 (3) GM00621 (3)	GM13740 (1) GM13741 (1)	707
NARP	Osteosarcoma	HGA13 (3) HPC7 (1) H1959 (1)	239 (1) 261 (1)	1439
Rho-zero	Osteosarcoma	HGA13 (3) HPC7 (1) H1959 (1)	143B206 rho-zero (2)	1844

TCAGAGCTCCCTGG-3′ and reverse 5′-CTACTTCCCT GGTCAGGCGC-3′; ATF4 forward 5′-AAGCCTAGGT CTCTTAGATGATTAC-3′ and reverse 5′- CAACCTGG TCGGGTTTTGTTAAAC-3′; β-actin forward 5′-ACGG CATCGTCACCAACTGG-3′ and reverse 5′-TTCATGA GGTAGTCAGTCAGG-3′; and GAPDH forward 5′-CC CCTGGCCAAGGTCATCCATG-3′ and reverse 5′-CAG TGAGCTTCCCGTTCAGCTC-3′. PCR was carried out using a Roche LightCycler (Indianapolis, IN), denaturation at 95 °C for 0 s, annealing at 60 °C for 8 s, and extension at 72 °C for 15 s for 40 cycles. Samples were quantified by generating a standard curve using cDNA transcribed from brain RNA (Ambion, Austin, TX). Student's *t*-test was used to determine significant results.

3. Results

3.1. Cells bearing mitochondrial disease mutations share transcriptional alterations

The study design used was that 22 groups of mutants versus controls all microarrayed on the U95Av2 chip format were compared for significantly altered transcripts at the $p \leqslant 0.05$ criterion, and a list was made of these significantly altered genes from each of the 22 experiments (Table 1). Our null hypothesis is that the transcriptional consequences of each mitochondrial disease and within each cell type are independent among each group. We derived the random expectation by two methods, firstly by a Poisson distribution, and secondly by an empirical randomization method, i.e. randomizing mutant and control chip within each of the 22 groups – and these methods agreed very well (Fig. 1).

For the Poisson method, the median number of transcripts significantly altered per group was 529, and the number of genes represented on each chip was 12,559, so our random expectation for overlap between groups is $529/12{,}559 = 4.2\%$ for any particular gene, i.e. lambda in the Poisson equation is 0.042, and the random expectation for sharing transcripts among groups is shown in Fig. 1. There was a huge excess of transcripts significantly shared among groups over the random Poisson expectation, for example 5- and 100-fold in the 1 and 2 transcripts shared categories, but steadily increasing, such as 8000- and 700,000-fold in the 3 and 4 transcripts shared categories, and 30 million- and 9 billion-fold in the 5 and 6 transcripts shared categories (Fig. 1).

Shared Transcripts	Poisson Expectation	Randomized Expectation	CTL vs MUT Observed	Fold Excess over Poisson
1	506	554	2515	4.97E+00
2	10	72	1696	1.70E+02
3	0.13	9	1125	8.65E+03
4	0.001	3	707	7.07E+05
5	0.000013	0	387	2.98E+07
6	9.20E-08	0	232	2.52E+09
7	5.50E-10	0	100	1.82E+11
8	2.76E-12	0	31	1.12E+13
9	5.60E-17	0	15	2.68E+17
10	2.10E-19	0	5	2.38E+19
11	7.50E-22	0	0	0.00E+00

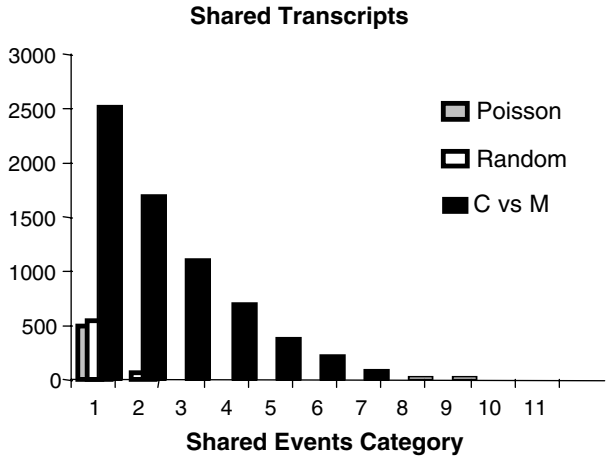

Fig. 1. An excess of shared transcripts among mitochondrial mutant vs. control groups versus random expectation. The table at left and the graph at right show the expected number of significantly altered transcripts whose identity is shared among groups under two random models, i.e. the Poisson (gray bar) and an empirical randomization method (white bar), compared with the actual number of sharing events of the identical transcript observed (black bar).

A second, empirical way to determine a random expectation was to randomize mutant chips vs. control chips within groups, and to then compare how frequently transcripts were shared among the groups. Both the Poisson and the empirical randomization methods agreed very well with each other. There was a small excess of between-group sharing observed in the empirical randomization method versus the Poisson methods, and this is likely the result of using some controls in common among groups. It is also clear from the randomization method that there are hundreds-fold more shared transcripts observed among the mutant vs. control groups than expected at random, and in the 1, 2, 3, and 4 categories, there are 5-, 23-, 125-, and 235-fold more shared transcripts observed than in the randomized groups. And there are at least 100-fold excesses in shared transcripts in the 5, 6, and 7 categories. Thus, from both the Poisson and the empirical randomization methods, there is a huge and significant excess of transcripts shared among mitochondrial disease. This huge and significant excess of transcripts shared among mitochondrial disease cannot be explained only by a common transcriptional response to mitochondrial disease in one cell type, because the maximum number of mutant vs. control comparisons within a cell type was 5, and several transcripts are significantly shared in 10 mutant vs. control comparisons. Thus there are shared transcriptional responses to mitochondrial disease that extend across multiple mitochondrial diseases and across multiple cell types.

3.2. Mitochondrial disease is transcript-inhibitory in extent and ratio

We counted the number of times transcripts were significantly altered up or down and shared among at least 3 of the 22 groups, which occurred for 2603 of the 12,559 transcripts. The median of the median fold change values of 2603 transcripts was −1.3, i.e., net-negative, supporting the view that the overall effect of mitochondrial disease on the transcriptome is net-negative.

Also, net down-regulations were much more common than net up-regulations. In 1620 of the 2603, the effect of mitochondrial disease was net-negative, in that more transcripts were down-regulated than up-regulated compared to the 746 transcripts which were net-positive; and there were 237 net neutral alterations, in which upregulations balanced downregulations. Thus, shared inhibitory changes outnumbered stimulatory changes by 2 to 1, and by this criterion also the imprint of mitochondrial disease is inhibitory on the transcriptome.

3.3. Mitochondrial disease activates transcripts of ER stress, the unfolded protein response, and cell proliferation

Transcripts were ranked with respect to the number of times they were significantly activated in mutants vs. controls in the 22 comparisons of mutants vs. controls, and then by median fold change (Table 2). Top-ranked up-regulated transcripts segregated heavily into the categories of cell cycle and cell proliferation (13 and 9, respectively), and stress-related, endoplasmic reticulum, and unfolded protein response (8, 7, and 6, respectively). Other functional categories of up-regulated transcripts included transcription factors (6), histones (5), and cytoarchitecture, glycolysis, and hypoxia (3 each).

3.4. Mitochondrial disease inhibits transcripts involved in vesicular secretion, translation, synaptogenesis, oligodendrogenesis, and migration

Transcripts significantly inhibited by mitochondrial disease were ranked by most-shared and by the median amplitude of inhibition (Table 3). COUP-tf2 was the top-ranked down-regulated gene, being significantly down-regulated in 9 of the 22 mutant vs. control comparisons, suggesting that COUP-tf2 responds to mitochondrial dysfunction.

Table 2
Shared transcripts activated by mitochondrial disease

Transcripts shared and induced	Abbreviation	Shared upregulated	Median	Cell cycle	Cell proliferation	Stress	UPR	ER	Transcription factor	Histone	Cytoarchitecture	Glycolysis	Hypoxia	Other	Function
H2A histone family, member O	H2AA	6	2.1	+	+					+					Histone
Tubulin, α1, isoform 44	tuba1	6	1.6		+						+				Microtubule polymerization
Glycyl-tRNA synthetase	GARS	6	1.6												Charging of glycine-tRNA, Charcot-Marie-Tooth
Proline-4-hydroxylase	P4HA1	6	1.5			+	+	+					+		Collagen secretion, chaperone, ER, hypoxia
Glucose regulated protein, 58 kDa	GRP58/PDI	6	1.4			+	+	+							ER stress response, chaperone, complexed with PDI
Kynurenine-oxoglutarate transaminase	Kat1/CCBL1	6	1.2											+	Mitochondrial, kynurenine/quinolinate pathway
CCR4–NOT transcription complex	cnot4	5	1.6											+	Transcription, activates ubiquitin ligase
Karyopherin (importin) β2	TNPO1	5	1.6											+	Nuclear protein import
Glucose-regulated protein 75, mthsp7C	mot-2/GRP75	5	1.6			+								+	Mitochondrial protein stress chaperone
Ceramide glucosyltransferase	UGCG	5	1.6										+		Glycosphingolipid metabolism
c-MYC	MYC	5	1.5	+					+			+			Cell proliferation, induction of glycolysis
Chop/ddit3/GADD 153	GADD 153	5	1.5	+		+	+	+	+						Stress-induced transcription factor, UPR, blocks adipogenesis
Scavenger receptor class B	SCARB2	5	1.5											+	Lysosomal membrane glycoprotein, secretion, endocytotic recycling
Polymerase (DNA directed) sigma	POLS	5	1.5	+	+	+									Stress, sister chromatin cohesion/exchange, translesion synthesis
S-Adenosylmethionine decarboxylase	AMD1	5	1.5											+	Polyamine biosynthesis
Peroxiredoxin 4	PRDX4	5	1.4											+	Cysteine-based antioxidant
DDX21	DDX21	5	1.4	+										+	Required for rRNA processing
Quinolinate phosphoribosyltransferase	qprt	5	1.4											+	Quinolinate neurotoxin pathway
RNA cyclase	RTCD1	5	1.4		+										RNA splicing
CDC-like kinase 1	CLK1	5	1.4	+											Cell division cycle, phosphoinositide-stimulated
DNAJ protein family	DNAJB6	5	1.4			+	+	+							DNAJ homolog, hsp40, brain-specific chaperone, inhibits huntingtin aggregation
Kinesin heavy chain member 2	KIF2a	5	1.4								+				Neuron-specific microtubule depolymerizer
Glucosamine-6-phosphate isomerase	GNPDA1	5	1.4									+			Converts glucosamine-6-phosphate to fructose-6-phosphate
Forkhead box E2	FOXE1	5	1.4						+					+	Transcription factor involved in thyroid development/migration
Aldolase C, fructose-bisphosphate	ALDOC	5	1.3									+			Glycolysis, neuron-specific isoform, transcription is upregulated by MYC, cAMP
CDC10 homolog, Septin 7	Septin7	5	1.3	+	+						+				Actin binding, cell migration
VDAC	VDAC1	5	1.3											+	Mitochondrial voltage dependent anion channel
Transgelin	TAGLN1	5	1.3								+				Actin cross-linking, actin binding, muscle development
Origin recognition complex	ORC1L	5	1.2	+	+										Essential for initiation of DNA replication in human cells

(*continued on next page*)

Table 2 (*continued*)

Transcripts shared and induced	Abbreviation	Shared upregulated	Median	Cell cycle	Cell proliferation	Stress	UPR	ER	Transcription factor	Histone	Cytoarchitecture	Glycolysis	Hypoxia	Other	Function
VAMP-1/synaptobrevin	VAMP1	5	1.2											+	Vesicular secretion/synaptogenesis/ docking
TNF type 2 receptor associated protein	TRAF2/TRAP3	5	1.1			+	+	+						+	Adaptor, required for TNF downstream effects, participates also in UPR
BACH1	BACH1	5	−0.1						+						Heme-binding$_{TRAP3}$
Activating transcription factor 4	ATF4/CREB-2	5	−0.1			+	+	+	+					+	Unfolded protein response, induces UPR and CHOP
H1 histone family, member 2	HIST1H1C	4	4.9	+	+					+					Histone
Rag D protein	rragd	4	3.9	+						+				+	GTPase, nucleolar and ribosomal assembly
Purine-rich element binding protein A	PURA	4	2.0	+					+						Binds ssdna, functions in transcription and DNA replication
Bip/GRP78	GRP78/BiP	4	1.9			+	+	+					+		UPR, HSP70, induced by stress:heat-, glucose-, energy-, oxygen-deprivation
Histone H1,b2k	HIST1H2BK	4	1.8	+	+					+					Histone
Nr1d2	Nr1d2/BD73	4	1.8						+						Transcription factor stimulated by globin synthesis and NO
H2B histone family, member D	HIST1H2BN	4	1.7	+	+					+					Histone
				13	9	8	7	6	6	5	3	3	3		

COUP-tf2 functions in vesicular secretion (Bardoux et al., 2005), angiogenesis (Pereira et al., 1999; You et al., 2005), neurogenesis (Park et al., 2003), and neural pathfinding (Tripodi et al., 2004).

The top 40 genes were ranked by functional category (Table 4). The top-ranked category was (vesicular) secretion with 15 hits, followed by migration (13), signaling (10), lipid (9), oligodendrocyte-specific (4), GTP-binding (4), transcription factor (4), synaptogenesis (3), translation (2) and antioxidant (2). Thus, the most-shared and most-negative transcriptomal impact among the mitochondrial diseases studied are in these functional categories.

3.5. Confirmation of functional impact of mitochondrial disease Onto-Express

Table 4 was constructed by identifying the function of only the 'top 40' most-shared transcripts arranged by descending median fold change. Another objective method to determine if particular functional categories are overemphasized in microarray data is through use of Bioprocess function of Onto-Express, which categorizes each transcript with respect to the biological processes in which it functions, and then compares whether functional transcriptional clusters are more altered in mitochondrial disease than expected at random (Khatri et al., 2002). We performed Onto-express analysis on the top 2603 transcripts significantly shared at $p < 0.05$ in 3 or more of the 22 groups. Onto-Express analysis of the 2603 transcripts confirmed a statistically significant alteration of many of the functional clusters we had identified by inspection in Tables 2 and 3, e.g. protein synthesis, vesicular secretion, synaptogenesis, cell cycle, transcription factors and glycolysis (Table 4). Thus there is substantial agreement with respect to functional category affected among the top 80 shared transcriptional consequences of mitochondrial disease and dysfunction with the top 2603, in that protein synthesis, vesicular secretion, cell migration, cell adhesion, and synaptic transmission are significantly altered functional clusters.

3.6. Mitochondrial inhibition triggers transcriptional activators of the unfolded protein response, ATF4 and CHOP

The activation of transcripts of the unfolded protein response (UPR) observed in Table 2 suggested that mitochondrial inhibition, i.e. the likeliest common consequence of mitochondrial disease, might be sufficient for activation of UPR. Activation of the UPR occurs when ER function is perturbed (i.e. impaired protein folding or processing reactions) (Kaufman, 1999). Apoptosis can occur when ER stress is prolonged and involves genes like GRP78 and GADD153/CHOP (Oyadomari and Mori, 2004; Sherman and Goldberg, 2001). To test the dependence of induction of the unfolded protein response (UPR) on mitochondrial dysfunction, cells were inhibited with 0.1

Table 3
Shared transcripts inhibited by mitochondrial disease

Transcript	Abbreviation	Down-regulations	Median	Secretion	Migration	Signaling	Lipid	GTP-binding	Oligo-dendrocyte	Transcription factor	Synaptogenesis	Translation	Antioxidant	Other	Function
COUP-TF2	coup-tfll	9	−3.0	+	+		+			+					Lipid metab., neurogenesis, angiogenesis
Dihydropyrimidinase-like 2	dpysl-2	8	−1.8	+	+	+			+						Neuro-migration, vesic. secretion, oligodendrocytic
Tetraspan 3	tspan3	8	−1.7		+				+		+				Neuro-migration, adhesion, oligodendrocytic
RAB11A	rab11a	8	−1.7	+	+	+		+							Vesic. secretion, endosomal recycling, neuromigration
Prot. Kinase inhibitor	pkig	8	−1.6			+									Inhibitor of protein kinase A
Protein phosphatase 2	PPP2R5C	8	−1.5			+									Cytosolic and mitochondrial phosphatase
ATPase, H+ transporting,	ATP6V1F	8	−1.4	+											Vesicular secretion
Niemann-Pick disease	NPC2	8	−1.3	+			+								Cholesterol transport, vesicular secretion
Translation initiation factor	EIF3S8	8	−1.3					+				+			Translation initiation
β-Tubulin	tubb	7	−2.8		+	+		+						+	Cytoarchitectural, signaling, GTP binding
Tissue factor pathway inhibitor	tfpi	7	−2.8	+										+	Anticoagulatory protein
CAAX box 1	cxx1	7	−1.8	+			+								Farnesylated protein
Dihydroceramide desaturase	degs1	7	−1.7	+	+	+	+		+						Dihydroceramide desaturase
Peroxiredoxin 2	prdx2	7	−1.7										+		Antioxidant
Phosphatidylinositol kinase	PIP5K2B	7	−1.5			+	+								Generates phosphatidylinositol 4,5-bisphosphate
GTPase activating protein 1	IQGAP1	7	−1.5		+			+			+				Cell migration, synaptogenesis
Biliverdin reductase A	BLVRA	7	−1.5											+	Heme catabolism, caveolae/ER association
Aflatoxin aldehyde reductase	AKR7A2	7	−1.5	+										+	Golgi aldehyde, ketone reductase
MILL septin-like fusion	MSF-A	7	−1.5		+									+	Microtubular, cell migration, vesic. trafficking
PHD zinc finger protein	PHF10/XAI	7	−1.4							+				+	Transcription factor
Diazepam binding inhibitor	DBI/ACBP	7	−1.4				+								Lipid metabolism,binds Carnitine palmitoyltransferase
Inositol 1,3,4-triphosphate kinase	ITPK1	7	−1.4			+	+								Mitochondrial kinase
Protein kinase C-like 1	PKN1	7	−1.3			+	+								Protein kinase
Bromodomain containing 3	brd3	7	−1.3							+					Transcription factor
Translation initiation factor 4B	eif4b	7	−1.3									+			Translation initiation
Lysosomal ATPase	ATP6V1E1	7	−1.2	+											Vesicular secretion
IGFBP5	IGFBP5	6	−8.3	+		+								+	Secreted, binds igf-1, important in bone growth
Paraoxonase 2	PON2	6	−4.3								+		+		Antioxidant, coexpressed acetylcholine receptor
Chemokine ligand 2	ccl2/MCP-1	6	−3.3	+	+										Secreted glycoprotein, migration
Chemokine receptor 4	CXCR4	6	−2.7		+										Chemokine receptor, cell migration
Actin smooth muscle, aorta	ACTA2	6	−2.7		+									+	Cell motility/structure/muscle
Hematopoietic homeobox	HHEX	6	−2.6							+					Transcription, hematopoiesis
Caspase 8	casp8	6	−2.1											+	Apoptosis
Vascular Rab-GAP/TBC	TBC1D8	6	−2.1	+	+										Vacuolar atpase
α-2 Antiplasmin	SERPINF1	6	−2.0	+	+										Antiangiogenic, neuroprotective
RAB31/rab22	rab31/22b	6	−2.0	+			+	+	+						Oligodendrocytic, delivers lipid to sheath
				15	13	10	9	4	4	4	3	2	2	9	

Table 4
Onto-express confirmation of significantly altered functional clusters

Functional cluster	Hits	Possibility	*p*-value
Transcripts activated in 3 or more groups			
Protein biosynthesis	19	156	0.012
Regulation of cell cycle	17	155	0.01
Cell proliferation	16	189	0.04
Intracellular protein transport	13	101	0.01
Protein transport	13	88	0.02
Regulation of Pol II transcription	13	134	0.04
Endocytosis	6	48	0.04
ER to Golgi transport	5	12	0.01
Vesicle-mediated transport	5	24	0.01
Glycolysis	5	29	0.02
	112	936	
Transcripts inhibited in 3 or more groups			
Protein biosynthesis	50	156	1.14E-07
Regulation of cell cycle	40	155	0.02
Regulation of Pol II transcription	33	134	0.03
ATP-coupled proton transport	11	28	0.02
Microtubule-based movement	8	17	0.02
Translational elongation	7	12	0.02
Translational initiation	7	13	0.02
Microtubule polymerization	6	8	0.01
Regulation of cell migration	6	8	0.01
Regulation of cell adhesion	6	11	0.02
Regulation of DNA replication	5	6	0.02
Synaptic transmission	5	153	0.02
	184	701	

and 1 nM of the mitochondrial inhibitor rotenone, at which concentrations both the two best known transcriptional mediators of UPR, ATF4 and CHOP, were induced (Fig. 2). At these concentrations of rotenone cells had >90% viability at 18 h, i.e. their viabilities were indistinguishable from those of untreated cells. Thus, inhibition of mitochondria induces the UPR in the absence of cell death.

Interestingly, induction of UPR transcripts has recently been demonstrated both as a consequence of ischemic hypoxia in neurons (DeGracia and Montie, 2004) and also as a consequence of mitochondrial inhibition (Ryu et al., 2002; Kuruvilla et al., 2003).

3.7. Induction of cell cycle and histone genes by mitochondrial disease

Several cell-cycle related transcripts were induced in mitochondrial disease, including MYC, H2AA, CHOP, POLS, DDX2, CLK1, CDC10, ORC1L, HIST1H1C, RRAGD, PURA, HIST1H2BK, and H1H2BN. Our observation that Myc was induced by mitochondrial disease is consistent with that of Miceli and Jaswinski who observed MYC induction in three rho-zero cell lines (Miceli and Jazwinski, 2005). The observation of induction of multiple cell cycle genes was interesting, given the recent finding that ischemic hypoxia results in an activation of cell-cycle dependent genes in organisms, and this activation is necessary for hypoxic cell death (Katchanov et al., 2001; Bossenmeyer-Pourie et al., 2002).

3.8. Confirmation of decreased COUP-tf2 and glycoprotein m6a transcripts by Q-RTPCR

Q-RTPCR of selected transcripts was carried out on RNA isolated from control and mitochondrial disease lymphoblasts. A 4-fold down-regulation of COUP-tf2 transcript in disease lymphoblasts microarrayed vs. controls was confirmed (Fig. 3A). COUP-tf2 encodes a

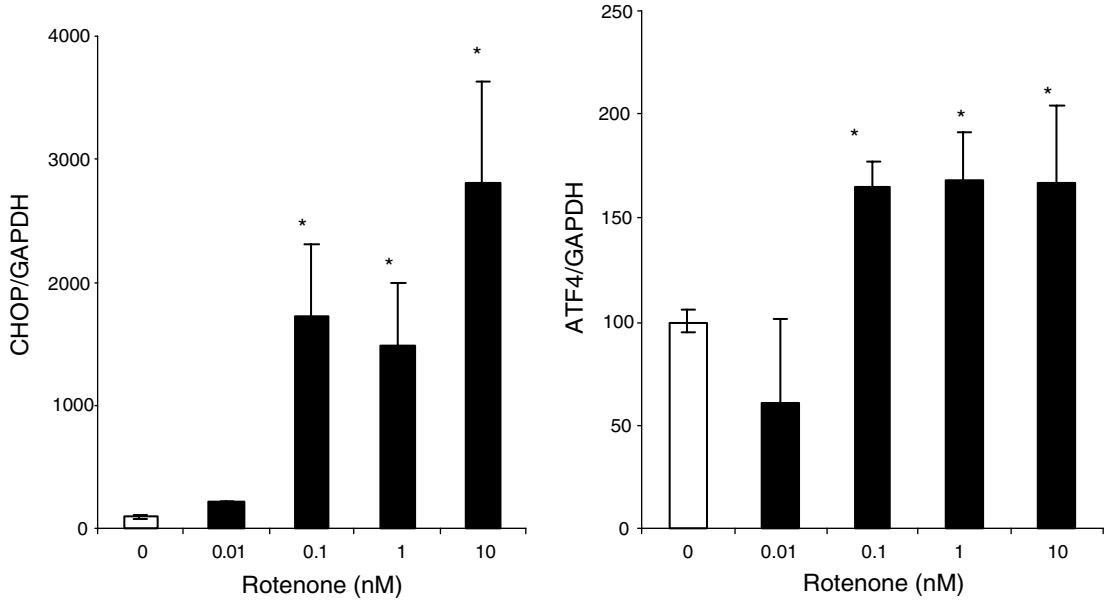

Fig. 2. Mitochondrial inhibition induces the UPR. Lymphoblasts were incubated with increasing doses of rotenone for 18 h, and CHOP and ATF4 transcript levels were assayed by Q-RTPCR and normalized to GAPDH expression. Means represent percent of untreated cells in at least three experiments and error bars represent SEM. $^{*}p < 0.05$.

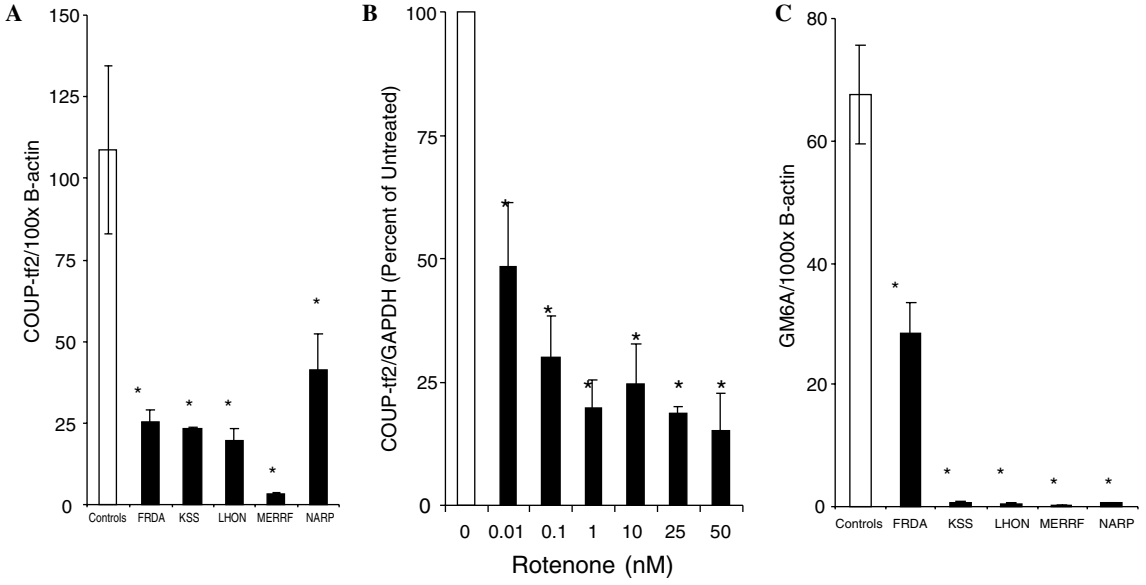

Fig. 3. Downregulation of COUP-tf2 by mitochondrial disease or inhibitors in lymphoblasts. (A) Quantitative RT-PCR was used to measure COUP-tf2 transcript levels from RNA isolated from control and disease lymphoblasts. Means represent at least three experiments and error bars represent SEM. $^{*}p < 0.05$. (B) Mitochondrial inhibition reduces COUP-Tf2 transcript levels. Lymphoblasts were incubated with the indicated concentration of rotenone for 18 h. Data shown represent the ratio of the treated per untreated samples. Means are representative of at least three experiments. Error bars represent SEM. $^{*}p < 0.05$. (C) Q-RTPCR of glycoprotein m6a (GPM6A) transcript levels in lymphoblasts.

transcription factor, knockouts of which are defective in vesicular secretion (Bardoux et al., 2005), angiogenesis (Pereira et al., 1999; You et al., 2005) and neural migration (Tripodi et al., 2004). A significant decrease in COUP-tf2 protein expression in nuclear extracts was also observed (data not shown). Both rotenone, a complex I inhibitor, and CCCP, a mitochondrial uncoupler, significantly inhibited COUP-tf2 transcript level (Fig. 3B).

Similarly, microarray data indicated that the GPM6A transcript (also known as glycoprotein m6a/EMA/DMβ) was strongly decreased. GPM6A encodes a proteolipid gene with substantial homology to oligodendrocyte-derived myelin that is expressed at the leading edge of neural growth cones (Werner et al., 2001). The 20-fold decrease of GPM6A observed by microarray was confirmed by Q-RTPCR in lymphoblasts (Fig. 3C). Antibodies to GPM6A interfere with neurite outgrowth in vitro (Yan et al., 1993), and decreased expression of GPM6A is correlated with decreased dendritic neurogenesis (Alfonso et al., 2004).

4. Discussion

4.1. There is a huge excess in shared transcriptional alterations among cells with mitochondrial disease over the random expectation

Even though KSS, MERRF, LHON, NARP, FRDA, and mtDNA depletion is caused by distinctly different mutations of the mitochondrial or nuclear genome, the mitochondrial defect is the primary cause of the disease. Using microarray, we asked which features of the nuclear response to these primary mitochondrial defects are shared among 5 mitochondrial diseases in 9 cell types. We observe a much higher sharing of many transcripts than expected if each were completely independent diseases with random effects on the genome and among cell types (Fig. 1). For example, 5 transcriptional alterations were shared among 10 of the 22 experiments, an excess of 2×10^{19}-fold over expectation. Even by using the more conservative empirical randomization method, our observed shared transcripts in 4, 5, 6, 7, and 8 experiments are each 100-fold higher than expected at random. Thus there is substantial sharing of the transcriptomal consequences of mitochondrial disease, both within and among diseases and cell types.

4.2. Mitochondrial disease is transcript-inhibitory overall

The shared transcriptional impact of mitochondrial disease was about twice as inhibitory as stimulatory. Similarly, the amplitude of the median fold change was larger among the down-regulated genes than the up-regulated genes, i.e. there are not only more genes inhibited, they are inhibited to a greater extent. Thus, mitochondrial disease has a net inhibitory effect on the transcriptome.

4.3. Mitochondrial disease and mitochondrial inhibition activate ER stress and unfolded protein response transcripts

A prominent functional cluster activated in cells with mitochondrial disease includes several members of the UPR (Rutkowski and Kaufman, 2004; Schroder and Kaufman, 2005). For example, ATF4, CHOP/DDIT3/GADD153, GRP58, GRP78/Bip, GRP75/mortalin-2/MTHSP70, and DNAJB6, are all significantly shared and

up-regulated, and are members of the UPR. The UPR has recently been implicated in a large number of neurological and neurodegenerative diseases (Paschen and Frandsen, 2001). The microarray results support the notion that mitochondrial disease causes ER stress and induction of the UPR.

This hypothesis was confirmed by inhibition of mitochondrial function with rotenone, in that rotenone specifically induces ATF4 and CHOP, the central transcription factors that regulate UPR (Rutkowski and Kaufman, 2004). A previous report demonstrated that overexpression of an unfolded protein in mitochondria activated the CHOP transcript (Zhao et al., 2002), but that report did not observe the activation of ATF4, nor of the endoplasmic reticular stress transcripts of the UPR (e.g. GRP78/BiP, GRP58, PRHA1, DNAJ) that we did observe in this study. Other reports have suggested that the neurotoxin MPP+ (which is also a Complex I inhibitor) induces the UPR, although the authors did not attribute the induction of UPR to the mitochondrial inhibition (Ryu et al., 2002). Also, another group has shown that depletion of mitochondrial membrane potential induces both the UPR and cell cycle genes (Kuruvilla et al., 2003). Data presented here indicate that activation of the UPR is both a common consequence of mitochondrial disease, as it is in other neurological diseases, and is also a specific consequence of mitochondrial inhibition.

4.4. Mechanistic connections between mitochondria and ER stress

The combined microarray data and rotenone tests support the idea that mitochondrial disease and dysfunction both activate the UPR, presumably through generation of ER stress. It has been shown by others that activation of the UPR gene product GRP78/Bip (whose transcript we observe is induced in mitochondrial disease) inhibits vesicular secretion, and we find vesicular secretion transcripts specifically inhibited (see below).

Mitochondria and ER are in close, functional contact (Rizzuto et al., 1998). There are at least three ways in which mitochondrial defects could generate ER stress, and thus trigger UPR and a defect in vesicular secretion. One way is that mitochondrial disease may increase mitochondrial generation of reactive oxygen species (ROS), for which there is substantial support in the literature (Wong et al., 2002; Rana et al., 2000; Rusanen et al., 2000; Wei et al., 2001; Geromel et al., 2001). A second possibility is that mitochondrial bioenergetic defects cause defects in ER Ca^{2+} signaling (Rizzuto et al., 1998; Brini et al., 1999), which may cause defects in professional ER proteins that are each strongly Ca^{2+} binding, and Ca^{2+} responsive. The fact that VDAC1 and MTHSP70, which are thought to reside at the mitochondrial–ER interface and control Ca^{2+} transients (Rizzuto R., personal communication), are among the most-shared and induced genes (Table 1) tends to support this idea of a defect in mitochondrial-to-ER signaling in mitochondrial disease.

A third possibility is that the mitochondrial defect causes a problem in mitochondrial lipid biosynthesis (such as the mitochondrial sphingolipid generated by DEGS1, dihydroceramide desaturase, which is significantly inhibited in 7/22 groups), which should cause a defect in proteolipid tagging in the ER, and a consequent increase in ER stress.

4.5. Induction of c-myc and ribosomal and cell cycle genes

Besides UPR, c-Myc, cell cycle and ribosomal genes were also prominently induced (Table 2). The protooncogene Myc was significantly induced by a median value of 1.5-fold in 5 groups with mitochondrial disease. The upregulation of Myc is known to drive the cell through the cell cycle, and thus may be responsible for the upregulation of several cell-cycle related genes in mitochondrial disease, including histone H2AA, RTCD1, CLK1, CDC10, ORC1L, histone H1C, RRAGD, PURA, histone H2BK, and histone H2BN. Deficits of mitochondrial function were recently shown to induce Myc (Miceli and Jazwinski, 2005), and Myc induction has recently been demonstrated to induce ribosomal RNA synthesis, consistent with observing RRAGD overexpressed in mitochondrial disease (Grewal et al., 2005). Also, it was demonstrated that ischemic hypoxia in whole animals induces cell cycle genes, and that the induction of these cell cycle genes is often necessary for cell death (Katchanov et al., 2001; Bossenmeyer-Pourie et al., 2002). Thus, induction of cell cycle genes appears to be a general (although still somewhat paradoxical) consequence of mitochondrial dysfunction that is the result of ischemic hypoxia or genetic mutations.

4.6. UPR induction is known to inhibit vesicular secretion and protein synthesis

The activation of the UPR and ER stress transcripts has the potential to explain at least two functional categories of transcripts observed to be inhibited in mitochondrial disease, i.e. vesicular secretion transcripts, and protein synthesis, because UPR induction is known to inhibit these pathways (Rutkowski and Kaufman, 2004; Watson et al., 2003). In the UPR, unfolded proteins in the ER activate the ER-localized PERK Kinase, which phosphorylates (and thus activates) ATF4, which translocates to the nucleus, inhibits transcripts related both to amino acid production and protein synthesis, and inhibits vesicular secretion; also, overexpression of GRP78/BiP inhibits vesicular secretion (Watson et al., 2003; Rutkowski and Kaufman, 2003).

We observe protein synthesis as a major functional category impacted by mitochondrial disease by Onto-Express analysis of the 2603 transcripts shared in 3 or more of the 22 comparisons (Table 3), and by the 'top 40' method the top-scoring and most-shared inhibited members are the translation initiation factors EIFS38 and EIF4B (Table 2). Furthermore, many most-shared inhibited transcripts either participate in the neural vesicular secretion of proteins or lipids (Rab11a, DPYSL-2, TSPAN3, ATP6V1F,

NPC2, MSF-A, ATP6V1E1, and Rab31/22b), or are the secreted cargo of that machinery (TFPI, IGFBP5, PON2, and CCL2) as shown in Table 2.

4.7. Transcripts involved in vesicular secretion and protein synthesis, cell synaptogenesis, and migration are specifically downregulated in cells with mitochondrial disease

The most-shared and down-regulated transcripts cluster into functional categories that include members of the vesicular secretion pathway, or proteins that are transported by the vesicular secretion pathway. These include COUP-tf2, Rab11a, and Rab31/22, two lysosomal ATPases, Niemann-Pick C2, TBC1D8, TFPI, and DPYSL-2.

In addition to their function in vesicular secretion, some others of this category have also been shown to be involved in cellular migration, i.e. COUP-tf2, DPYSL-2, tetraspan 3, Rab11a, β-tubulin, lipid desaturase, IQGAP, chemokine CCl2, and chemokine receptor CxcR4. Since cellular migration is dependent upon the process of vesicular secretion both for secreting cell migratory signals into the ECM, as well as for interpreting them (Bershadsky and Futerman, 1994), it is not unreasonable for a defect in vesicular secretion to cause a defect in cellular migration, and for these processes to be co-regulated.

Many of the above-mentioned genes function at the synapse, and tspan3, IQGAP1, and PON2 are known to have a specific synaptic localization.

4.8. Downregulation of COUP-tf2 is a prominent feature of mitochondrial deficiency

COUP-tf2 was the most-shared and most-downregulated transcript we observed among the mitochondrial diseases analyzed. COUP-tf2 has been recently shown to function in vesicular secretion (Bardoux et al., 2005), angiogenesis (Pereira et al., 1999; You et al., 2005), neurogenesis (Park et al., 2003), and neural pathfinding (Tripodi et al., 2004). Although COUP-tf2 is known to regulate many genes primarily through repression (Zhou et al., 2000), COUP-tf2 is also known to specifically regulate the transcription of at least three nuclear-encoded mitochondrially targeted genes: mitochondrial medium chain acyl dehydrogenase (Maehara et al., 2003), which is essential for fatty acid oxidation, mitochondrial aldehyde dehydrogenase (Stewart et al., 1998; Chou et al., 1999), and mitochondrial ATP synthase (Jordan et al., 2003). The data show that the COUP-tf2's steady-state transcript level responds to mitochondrial inhibition at very low concentrations of the mitochondrial complex I inhibitor rotenone (10 pM) within 18 h, at doses of drug in which cell viability is >90%.

4.9. Oligodendrocyte-specific transcripts are downregulated in mitochondrial disease

One cell type that is especially dependent on lipid synthesis, cell-to-cell contact, synaptogenesis and migration is the oligodendrocyte, and so it was striking that two of the top 3 most-downregulated genes, DPYSL2 and tetraspan 3, which are significantly downregulated in 8/22 groups, are 'oligodendrocyte-specific' genes (Ricard et al., 2000; Tiwari-Woodruff et al., 2004). In addition, the dihydroceramide desaturase transcript, DEGS1, which makes ceramide, the precursor of sphingomyelin, a major component of the myelin sheath, was down in 7/22 groups. Similarly the oligodendrocyte-specific Rab31/22b was significantly down in 6/22 groups; Rab31/22b is specifically involved in vesicular transport in oligodendrocytes, and is thought to direct lipid to the myelin sheath (Rodriguez-Gabin et al., 2001). Also, the glycoprotein m6a transcript was down in 5/22 groups. Glycoprotein m6a shares features with myelin proteolipid produced by oligodendrocytes, and is expressed in oligodendrocytes (Werner et al., 2001), and is decreased by an average of 30-fold in mutant cells (Fig. 1).

4.10. Oligodendrocytic defects could explain puzzling features of mitochondrial disease

The observation of a preferentially negative impact of mitochondrial deficiency on lipid biogenesis, vesicular secretion, and oligodendrocyte-specific genes suggests that demyelination could be an important pathophysiologic feature of mitochondrial disease, especially because myelin is transported by the vesicular secretion pathway. There is substantial support in the literature for the idea that mitochondrial disease and dysfunction causes demyelination, for the mitochondrial diseases complex I deficiency/Leigh syndrome (Bugiani et al., 2004), MELAS (Karppa et al., 2003), MERRF (Mizusawa et al., 1991), MNGIE (Bedlack et al., 2004), and complex IV deficiency (Uusimaa et al., 2003).

A mitochondrial-dependent deficiency in oligodendrocyte function could help to explain some previously difficult features of some mitochondrial diseases. For example, in individuals with LHON, there is an approximately 100-fold increase in the risk for multiple sclerosis (MS), a classic example of a demyelinating disease (Charlmers and Harding, 1996; Vanopdenbosch et al., 2000), which has never been explained. Similarly, in LHON it has clearly been documented that some patients recover their sight (Carelli et al., 2002), which would be impossible under a simple optic neurodegenerative hypothesis for the disease. Thus a mitochondrial-dependent deficiency in oligodendrocyte function has the potential to explain both the increased risk for MS in LHON, as well as the sporadic recovery that is observed. These data suggest that mitochondrial dysfunction preferentially causes defects in oligodendrocyte-specific genes, which may be primary causes of pathology.

4.11. Summary and prospects

The data show that there is a significantly shared 'transcriptomal imprint' of mitochondrial disease, which

implicates specific pathophysiological pathways: UPR activation, cell cycle activation, inhibition of vesicular secretion, inhibition of oligodendrogenesis, synaptogenesis and neural migration. We demonstrated that very low doses of mitochondrial inhibitors specifically activate the central regulators of UPR, ATF4, and CHOP, and inhibit COUP-Tf2, which functions in vesicular secretion, angiogenesis and neurogenesis. Others have shown that UPR induction and GRP78/BiP induction specifically decrease vesicular secretion and protein translation, and we observe inhibition of these functional categories by microarray. The data support the hypothesis that mitochondrial disease or dysfunction preferentially causes ER stress, which in turn triggers the unfolded protein response, which results in decreased translation and decreased vesicular secretion, resulting in decreased secretion of proteins involved in synaptogenesis, neural cell migration, and oligodendrocyte function. It is hoped that a clearer mechanistic understanding of mitochondrial disease will ultimately facilitate mechanism-based therapy for patients with mitochondrial diseases.

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