Title

Systems level analysis of transgenerational spermatogenic inheritance predicts biomarkers and underlying pathways.

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Abstract

Transgenerational spermatogenic inheritance of adult male acquired CNS gene expression characteristics has recently been discovered using a *Drosophila* systems model. In this novel mode of inheritance, transcriptomic alteration induced by the neuroactive drug pentylenetetrazole (PTZ) has been found to leak to future generations. Here, the available microarray gene expression data pertaining to CNS and/or testis of exposed F_0 and the resulting F_1 and F_2 generations has been pooled and analyzed in an unbiased manner at four levels, namely, biological processes and pathways, protein interactome networks, miRNA-targets, and microarray expression profile similarities. Enrichment for processes related to translation, energy metabolism, cell proliferation, cell differentiation, secretion, central nervous system development, germ cell development, gamete generation, wing development, nutrition etc. was observed. Also, ribosome, oxidative phosphorylation and, to a lesser extent, wingless signaling pathway showed overrepresentation. In the proteomic interactome map, the cell cycle gene Ras85D exhibited overinteraction. In miRNA-target network, the fly transgenerational genes showed overrepresentation of mir-315 targets. Transcriptomic matching revealed overlap of transgenerational set with genes related to epigenetic drug treatment, stem cells, Myc targets and miRNA targets. Many of the findings were consistent with the existing epigenetic evidence in complex mammalian traits. Converging evidence suggests that ribosomal RNA and proteins may serve as candidate biomarkers of transgenerational environmental effect. A compelling systems biology frame-work integrative of transgenerational epigenetic inheritance is suggested. Nutrient, circulating peptide hormone, Myc, Wnt, and stem cell signaling pathways constitute the frame-work. The

analysis has implications in explaining missing heritability in complex traits including common human disorders. The fly model offers an excellent opportunity to understand somatic and germline communication, and epigenetic memory formation and its retention across generations in molecular details.

Running title

Systems analysis of transgenerational inheritance

Introduction

Environmental exposures are known to influence health and disease. Emerging evidence increasingly implicates epigenetics as the mediator of environmental influences.[1-3] Since epigenetic changes have the potential to perturb gene expression in various cell types that constitute various tissues and organs, these changes are considered to provide a plausible basis for altered transcriptomic patterns associated with various diseases.[4,5] Epigenetic modifications in somatic cells can be mitotically inherited and thereby exert long-term effect on gene expression, a mechanism that is considered to underlie disease risk secondary to prenatal and early postnatal environmental exposures.[6-9] Importantly, increasing evidence suggests that epigenetic modifications may also be meiotically heritable and passed on to future generations.[2,10-13] Reported instances of inheritance of epigenetic transgenerational phenotype have however been limited to environmental exposures during embryonic and adult gonadal development.[14,15] Possibility remains that adult exposures affect gametogenesis and cause reprogramming of the germline.[14] Although instances of epigenetic effects on gametogenesis are reported, transgenerational inheritance of environment-induced adult phenotype has been a completely unknown phenomenon.[14,16-20] Startlingly, a systematic search conducted recently using a novel *Drosophila* transcriptomic model of brain plasticity has led to the discovery of a novel mode of inheritance in which adult male acquired CNS gene expression characteristics exhibit transgenerational spermatogenic inheritance.[21] Epigenetic codes play a crucial role in neural plasticity.[22,23] Availability of a *Drosophila* transcriptomic model of PTZ induced long-term brain plasticity described recently[24] actually motivated the search[21] for transgenerational spermatogenic inheritance. In the PTZ model, chronic drug treatment of

adult males causes alterations in CNS transcriptome.[24] To examine if PTZ induced gene expression changes are transgenerational inherited, CNS transcriptomic profiles were generated from F_1 adults after treating F_0 adult males with PTZ and from F_2 adults resulting from a cross between F_1 males and normal females.[21] Strikingly, microarray clustering showed F_1 male profile most similar to F_1 female and F_0 male profile to F_2 male. Differentially expressed genes in F_1 males, F_1 females and F_2 males showed statistically significant match with PTZ regulated genes.[21] In an unbiased approach to search for physical evidence of a possible spermatogenic mechanism, microarray expression profiles of adult testis from F_0 and F_1 males were analyzed.[21] Further striking, clustering of CNS and testis profiles and enrichment analysis of differentially expressed gene sets provided evidence of a spermatogenic mechanism in the transgenerational event.[21]

The discovery that gene expression phenotype acquired by an adult can be transmitted to future generations has obvious implications in human health and evolution. Recent epidemiological evidence indeed supports existence of sex-specific, male line transgenerational responses in humans.[25] Considering the importance, the phenomenon detected in *Drosophila* needs to be analyzed further for developing a systems level understanding of the mechanisms involved. Here, the available gene expression data has been analyzed in an unbiased manner using systems level tools. The available data pertaining to CNS and/or testis of exposed F_0 and the resulting F_1 and F_2 generations has been pooled and analyzed at four levels, namely, biological processes and pathways, protein-protein interaction networks, miRNA-target networks, and microarray expression

profile similarities. The analysis suggests potential mechanisms underlying spertmatogenic transmission of environmental effects in soma across generations.

Results and discussion

Biological Process and pathway enrichment

Gene ontology (GO) based analysis showed enrichment of translation in upregulated genes, whereas a variety of other processes were overrepresented in the downregulated set (**Table 1**; for a complete list and details, see **Supplementary Table 1**). Upregulation of translation was remarkable considering that overexpression of rRNA transcription was observed in the F_2 progeny of the original experiment.[21] Processes related to central nervous system development, germ cell development, neurogenesis, gamete generation, transcription, calcium signaling, nutrition, energy metabolism, wing development etc. showed enrichment in downregulated gene set. At pathway level, ribosome (**Figure 1**), oxidative phosphorylation (**Figure 2**), and Wnt (wingless) signaling (**Figure 3**) showed overrepresentation.

Nutrient sensing signaling pathway is known to encompass rRNA transcription from *Drosophila* to man.[26-29] Besides growth regulators, epigenetic modifications also control rRNA transcription across species.[30-37] Ribosomal components are also suggested to be involved in gene expression including epigenetic mechanisms in higher eukaryotes including *Drosophila*.[38-41] Environmental influences including nutritional factors are considered to underlie various known instances of transgenerational epigenetic inheritance of phenotypes such as metabolic syndrome, type II diabetes, obesity,

cardiovascular disorders, cancer, psychiatric conditions, longevity etc..[42-53] Further, differential expression or epigenetic modification of genes encoding ribosomal components has been associated with many diseases including cancer, Alzheimer's, and type II diabetes.[54-59] Reduced dosage of genes encoding ribosomal proteins has also been associated with a diverse collection of phenotypes across species.[60,61] Notably, epigenetic modification of rRNA genes besides others has been implicated in environmental factor induced transgenerational phenomena.[53] Given the above, the biological process enrichment analysis suggested that nutrient sensing, energy metabolism and growth regulation might possibly be involved in transmission of environmental influences. This is consistent with the earlier[21] demonstration of differential rRNA expression in the fly transgenerational experiment.

Protein interactome analysis

Transgenerational genes were next overlaid on to *Drosophila* proteomic interaction network to identify, if any, overinteraction. The gene CG9375 encoding Ras oncogene at 85D (Ras85D) was found to overinteract within the transgenerational gene set (**Figure 4**; for a complete list and details of the analysis, see **Supplementary Table 2**). It is known that endogenous Ras85D is required to maintain normal levels of the oncogene dMyc in *Drosophila*.[62] It has also been demonstrated that Myc binds to specific consensus elements located in human rDNA and associates with the Pol I-specific factor SL1.[63] Further, the presence of Myc at specific sites on rDNA has been found to coincide with the recruitment of SL1 to the rDNA promoter and with increased histone acetylation.[63] Myc is a known regulator of rRNA synthesis and ribosomal biogenesis in *Drosophila* as

well as in mammalian species.[30,35,39,63,64] Stimulation of rRNA synthesis by c-Myc has been proposed as a key pathway driving cell growth and tumorigenesis.[63] The growth effects of dMyc in *Drosophila* wing development require *de novo* rRNA synthesis.[64] The growth and proliferation regulators including Myc are reversibly acetylated or deacetylated by histone acetyltransferases or histone deacetylases (HDACs), respectively.[30] This connects activity of these proteins to chromatin-modifying enzymes.[30] Furthermore, Myc control of ribosome biogenesis has been found to be under nutritional control in *Drosophila*.[26] The Ras family members including Ras85D are known to affect cell fate and cell adhesion via the Raf/MAPK pathway.[62] Besides, regulation of dMyc levels by Ras85D has been suggested to be critical for wing development in *Drosophila*. The cell cycle protein Ras85D is also involved in signaling that promotes specification of photoreceptor neurons. It is known that in addition to coordinating cell growth and division through a transcriptional program that involves both RNA polymerase (Pol) II- and Pol III-transcribed genes, Myc also directly enhances Pol I transcription of rRNA genes.[63] Considering overrepresentation of processes related to ribosome, growth, wing development, nutrition, phototransduction, MAPK pathway, phototransduction etc. (**Table 1**; **Supplementary Table 1**), the interactome analysis suggested a possible role of Myc and Wnt signaling in the transgenerational event.

miRNA-target overrepresentation

Next, transgenerational genes were overlaid on to *Drosophila* miRNA-targets map. The genes were found to be enriched for targets of mir-315 (**Figure 5**; for a complete list of targets and details of the analysis, see **Supplementary Table 3**). Importantly, mir-315

has earlier been found to be a strong and specific activator of Wnt signaling in *Drosophila*.[65] The epigenetic drug valproic acid, an HDAC inhibitor, is known to regulate genes belonging to various pathways including ribosomal proteins, calcium signaling, wg signaling, MAPK signaling, focal adhesion, cell cycle etc.[66] Also, a recently isolated protein fraction from plant source that shows HDAC inhibitor activity has been found to contain ribosome-inactivating proteins and to inhibit Wnt signaling.[67] Importantly, exposure of the endocrine disruptor vinclozolin during gonadal sex determination, that promotes prostate disease phenotype across generations in rats, has been found to transgenerationally cause in the prostate differential expression of genes related to various pathways including calcium signaling and Wnt signaling.[68] Cumulatively, the above analysis supported a possible role of Wnt signaling in epigenetic inheritance.

Transcriptomic profile similarities

Finally, the fly transgenerational profiles were compared with other transcriptomic profiles in an unbiased manner. For this, a database of mammalian profiles was used. The mammalian homologs of fly genes were retrieved (for a list of homologs, see **Supplementary Table 4**) and the database was queried for statistically overrepresented profiles. This led to the identification of several enriched profiles (for a complete list and details of the analysis, see **Supplementary Table 5**). Many of the enriched profiles were related to epigenetic drug treatment, stem cells, miRNA targets and Myc-targets etc. (**Table 2**). The result was striking. For example, whereas the cytosine analog 5-aza-2-deoxycytidine (5azaC) is a widely used DNA demethylating agent, the HSP90 inhibitor

17-allylamino-17-demethoxygeldanamycin (17AAG) to influence expression of Myc-regulated mRNAs as well as chromatin associated proteins including heterochromatin protein 1, histone acetyltransferase 1, and histone arginine methyltransferase PRMT5.[69] The matching analysis surprisingly revealed similarity between fly transcriptome and several stem cell related profiles. Given the similarity between *Drosophila* and mammalian stem cell biology[70], the above evidence was striking. For example, Wnt signaling is known to regulate function and development of neural stem cells (NSCs) throughout an individual's lifetime.[71] Further, the inter- and intra-cellular molecular cascades in soma and germline stem cells (GSCs) in *Drosophila* are considered to be similarly affected by environmental factors including nutrient sensing.[72] Evidence suggests that nutritional conditions regulate neuro-endocrine signal in the form of expression of insulin-like peptides in specific head neurons in *Drosophila*.[72] These peptides are secreted, transported and bind to GSC surface receptors to control cell division.[72] In addition to somatic cells, the insulin signaling is considered to play an essential role in both spermatogenesis and oogenesis in *Drosophila*.[73] The transgenerational spermatogenic effect in *Drosophila* relates to exposure to PTZ, a gamma-aminobutyric acid (GABA) receptor antagonist.[21] Interestingly, CNS circuits connecting GABA and insulin-like peptide, besides others, have been described in *Drosophila*.[74] An altered energy metabolism in GSCs has earlier been proposed.[75] Also, translational machinery including ribosomal proteins has been implicated in control of GSC maintenance and differentiation in *Drosophila*.[75] A unified view of regulation of diversity of stem cells, including somatic and germline stem cells as well as downstream germ cells, in both *Drosophila* and mammals has been argued, in which ribosomal

mechanisms play a crucial role.[75] Further, the transcription factor Myc is a known regulator of stem cells across species.[76-78] Besides, Wnt signaling is considered to play a role in defining GSC and other stem cell niche in *Drosophila*.[79,80] The fly transgenerational genes were also enriched in miRNA-targets conserved across species (**Table 2**). The miRNAs are known to act as essential intrinsic regulators of stem cell division rate and identity.[80] Evidence shows that miRNA-mediated translational regulation may control self-renewal of stem cells including GSCs in *Drosophila*.[80,81] The miRNAs are considered to play an important role in both spermatogenesis and neuronal stem cell function.[82,83] Cumulatively, as the information that will be transmitted transgenerationally is contained in the GSCs, the above analysis may suggest that neuro-endocrine control of stem cells mediate transgenerational epigenetic inheritance of acquired somatic characteristics. Some kind of CNS-gametogenesis axis maintained by a neuropeptide signal has earlier been proposed to explain the phenomenon.[21] The present analysis seems to support this hypothesis.

Integration of genetic and epigenetic inheritance would be ultimately needed to understand human diversity and to realize the goals of personalized and predictive medicine. A topical example that may underscore this need is that of missing heritability. Analyses using the recently available technique of genome-wide association have though identified nucleotide sequence variations in numerous genes in complex traits such as diabetes, obesity, heart diseases etc., the individual and cumulative effects of the genetic variations are so small that they simply can not explain the higher estimates of heritability known of these disorders.[84,85] Transgenerational epigenetic inheritance may explain the

disparity to some extent.[85] However, it is not yet clear how the epigenetic changes are remembered by the next generation. It has been anticipated that model organisms would be useful in seeking answer to such questions.[85] The systems level analysis presented here may offer an excellent frame-work to further dissect soma-germline cross-talk, and epigenetic memory formation and its retention across generations in molecular details.

Methods

Genes previously reported as differentially expressed in CNS of PTZ exposed males, F_1 male and female CNS, F_2 male and female CNS, and F_0 and F_1 testis[21,23] were all pooled together to examine for enrichment analysis. Names or IDs of fly genes or their 'homologene' homologs were retrieved using FLIGHT (http://www.flight.licr.org/). Gene ontology (GO) biological process enrichment and KEGG pathway enrichment were analyzed using DAVID (http://david.abcc.ncifcrf.gov/home.jsp).[86] Protein network was analyzed using BioGRID v 2.0 (http://www.thebiogrid.org/index.php).[87] Enrichment for miRNA-target genes were analyzed using EMBL's 2005 database (http://www.russell.embl-heidelberg.de/miRNAs/). The software platform Osprey v. 1.2.0 (http://biodata.mshri.on.ca/osprey/servlet/Index) was used for visualizing protein interactions and miRNA-target network. The L2L database (http://depts.washington.edu/l2l/) was used to identify matching mammalian profiles. KEGG (http://www.genome.jp/kegg/tool/color_pathway.html) was used for depicting genes in the pathways.

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Figure 1.

Ribosomal protein encoding genes in the fly transgenerational set. Location of up- and down-regulated genes in KEGG pathway is shown as red and green boxes respectively. Combined analysis for up- and down-regulated genes showed significant enrichment (*p*=3.3E-08, Benjamini adjusted) of ribosome pathway. Separate analysis showed more significant enrichment in upregulated (*p*=3.0E-08) than downregulated (*p*=3.0E-05) genes. Grey boxes indicate other *Drosophila* genes. White boxes indicate absence of fly genes in the KEGG pathway.

Figure 2.

Oxidative phosphorylation pathway genes in the fly transgenerational set. Location of up-, down- and both up- and down-regulated genes in KEGG pathway is shown as red, green and red-green hybrid boxes, in that order. Combined analysis for up- and down-regulated genes showed significant enrichment (*p*=3.2E-02, Benjamini adjusted) of oxidative phosphorylation pathway. Grey boxes indicate other *Drosophila* genes. White boxes indicate absence of fly genes in the KEGG pathway.

Figure 3.

Wnt signaling pathway genes in the fly transgenerational set. Location of up- and down-regulated genes in KEGG pathway is shown as red and green boxes respectively. Downregulated genes showed significant enrichment for Wnt signaling pathway at nominal (0.002), not Benjamini adjusted (0.14), *p* value. Considering overrepresentation of wing development related GO processes in the downregulated genes after multiple

testing correction (in Table 1 and additional file 1), Wnt signaling pathway may be considered as enriched. Grey boxes indicate other *Drosophila* genes. White boxes indicate absence of fly genes in the KEGG pathway.

Figure 4.

Overinteraction of CG9375 (Ras85D) in fly transgenerational gene set. The within group protein-protein interaction subnetwork is shown. The top four highly interacting genes in the transgenerational set consisting of both up- and down-regulated genes were CG15218, CG3936, CG9375, and CG11525. Compared to genomewide network, all four showed significant overinteraction at nominal *p* value, p=0.024, 0.018, 0.00076, and 0.022, in that order. After Bonferroni correction for multiple comparison, only CG9375 (p=0.003) remained significant. In the subnetwork, all genes except CG2956 (twi) are CNS specific. The CG2956 represent both CNS and testis profiles. For details of interaction analysis and gene names and IDs, see Supplementary Table 2.

Figure 5.

Enrichment of mir-315 targets in fly transgenerational gene set. The within group miRNA-target subnetwork is shown. The top two miRNAs with highly enriched targets in the transgenerational set consisting of both up- and down-regulated genes were mir-277 and mir-315. Compared to genomewide targets, both miRNAs showed significant enrichment of targets at nominal *p* value, p=0.029, 0.014, in that order. After Bonferroni correction for multiple comparison, only mir-315 (p=0.028) remained significant. In the subnetwork, all genes exclusively represent CNS profiles except CG11390 (PebIII)

CG14007 and CG6784. Whereas CG11390 represent both CNS and testis, CG14007 and CG6784 represent only testis. For details of miRNA-target analysis and gene names and IDs, see Supplementary Table 3.

Table 1. Enriched biological processes in fly transgenerational gene set. Only a partial list is shown here. Complete list is provided in Supplementary Table 1.

GO_Term	Fold change	P Value
Upregulated		
GO:0006412~translation	3.3	0.001
Downregulated		
GO:0015031~protein transport	1.7	6.16E-10
GO:0046903~secretion	1.9	9.44E-09
GO:0000003~reproduction	1.5	1.77E-08
GO:0051179~localization	1.2	6.74E-08
GO:0030154~cell differentiation	1.3	7.30E-08
GO:0019226~transmission of nerve impulse	1.7	1.11E-07
GO:0006897~endocytosis	1.7	9.10E-07
GO:0007276~gamete generation	1.5	1.07E-06
GO:0001505~regulation of neurotransmitter levels	2.0	1.39E-06
GO:0007267~cell-cell signaling	1.6	1.92E-06
GO:0007268~synaptic transmission	1.7	5.91E-06
GO:0000902~cell morphogenesis	1.4	1.23E-05
GO:0007601~visual perception	2.1	1.43E-05
GO:0009791~post-embryonic development	1.4	1.82E-05
GO:0006810~transport	1.2	4.67E-05

GO:0006119~oxidative phosphorylation	1.8	6.78E-05
GO:0007015~actin filament organization	2.1	8.27E-05
GO:0007399~nervous system development	1.4	1.47E-04
GO:0048489~synaptic vesicle transport	2	1.69E-04
GO:0007281~germ cell development	1.8	2.91E-04
GO:0000165~MAPKKK cascade	2.0	3.46E-04
GO:0006928~cell motility	1.5	4.22E-04
GO:0007010~cytoskeleton organization and biogenesis	1.4	6.23E-04
GO:0009994~oocyte differentiation	1.8	6.84E-04
GO:0030029~actin filament-based process	1.7	7.43E-04
GO:0048599~oocyte development	1.9	8.61E-04
GO:0045165~cell fate commitment	1.5	0.001
GO:0007154~cell communication	1.2	0.001
GO:0007610~behavior	1.5	0.002
GO:0007602~phototransduction	2.3	0.002
GO:0010467~gene expression	1.1	0.002
GO:0016070~RNA metabolic process	1.2	0.002
GO:0007254~JNK cascade	2.2	0.002
GO:0016477~cell migration	1.5	0.003
GO:0031098~stress-activated protein kinase signaling pathway	2.1	0.003
GO:0007619~courtship behavior	2.3	0.005
GO:0006120~mitochondrial electron transport, NADH		

to ubiquinone	2.2	0.005
GO:0007417~central nervous system development	1.6	0.007
GO:0022414~reproductive process	1.5	0.007
GO:0048488~synaptic vesicle endocytosis	2.2	0.007
GO:0040007~growth	1.6	0.008
GO:0022008~neurogenesis	1.4	0.008
GO:0006911~phagocytosis, engulfment	1.5	0.008
GO:0006536~glutamate metabolic process	3.5	0.008
GO:0016319~mushroom body development	2.2	0.01
GO:0019722~calcium-mediated signaling	2.3	0.01
GO:0007618~mating	1.9	0.01
GO:0048024~regulation of nuclear mRNA splicing, via spliceosome	1.9	0.01
GO:0008360~regulation of cell shape	1.7	0.02
GO:0012501~programmed cell death	1.4	0.02
GO:0051301~cell division	1.4	0.02
GO:0006909~phagocytosis	1.4	0.02
GO:0048699~generation of neurons	1.3	0.02
GO:0048667~neuron morphogenesis during differentiation	1.4	0.03
GO:0001700~embryonic development via the syncytial blastoderm	1.5	0.03
GO:0006366~transcription from RNA polymerase		

II promoter	1.3	0.03
GO:0006457~protein folding	1.5	0.03
GO:0007420~brain development	1.7	0.03
GO:0042981~regulation of apoptosis	1.6	0.03
GO:0030182~neuron differentiation	1.4	0.03
GO:0019098~reproductive behavior	1.8	0.03
GO:0031667~response to nutrient levels	2.6	0.03
GO:0006887~exocytosis	1.6	0.04
GO:0006914~autophagy	2.6	0.04
GO:0016265~death	1.4	0.04
GO:0007613~memory	2.2	0.05
GO:0007317~regulation of pole plasm oskar mRNA localization	2.4	0.05
GO:0006350~transcription	1.2	0.05
GO:0007584~response to nutrient	3.3	0.05
GO:0040008~regulation of growth	1.7	0.05
GO:0000278~mitotic cell cycle	1.3	0.05

p values shown are after Bonferroni correction

Table 2. Enriched mammalian profiles in fly transgenerational gene set. Only a subset of enriched profiles is shown. For details, see Supplementary Table 5.

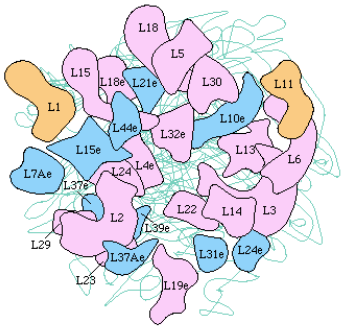
Description	**Fold change**	**Binomial P-value**
Downregulated		
Enriched in mouse neural stem cells, compared to differentiated brain and bone marrow cells	4.97	1.41E-119
Enriched in mouse embryonic stem cells, compared to differentiated brain and bone marrow cells	4.41	1.02E-68
Down-regulated in human hepatoma cells following treatment with 5azaC	4.0	5.09E-53
Predicted human MicroRNA targets	3.35	2.00E-43
Predicted MicroRNA targets conserved across human, mouse and rat	3.96	9.78E-36
Enriched in mouse hematopoietic stem cells, compared to differentiated brain and bone marrow cells	2.95	5.38E-30
Down-regulated in human hepatoma cells following treatment with trichostatin A	3.1	7.52E-20
Downregulated by butyrate in SW260 colon carcinoma cells	4.05	2.73E-11
Enriched in mouse embryonic, neural and		

hematopoietic stem cells, compared to differentiated brain and bone marrow cells	4.35	1.79E-10
Downregulated by butyrate in SW260 colon carcinoma cells	4.91	2.65E-08
Myc-responsive genes reported in multiple systems	7.02	1.10E-06
Predicted MicroRNA targets conserved across human, mouse, rat, zebrafish and fugu	3.79	1.44E-06
Up-regulated in mouse hematopoietic stem cells and progenitors from fetal liver	2.17	4.36E-06
Downregulated by TSA in SW260 colon carcinoma cells	3.52	1.92E-03
Upregulated by butyrate in SW260 colon carcinoma cells	4.21	2.80E-03
Upregulated		
Enriched in mouse neural stem cells, compared to differentiated brain and bone marrow cells	4.79	1.10E-06
Up-regulated in human hepatoma cells following treatment with trichostatin A	5.14	1.29E-03
Down-regulated in human hepatoma cells following treatment with 5azaC and with both 5azaC and TSA	3.46	1.48E-03
Up-regulated in more than one of several human		

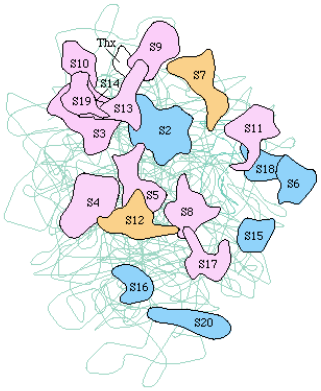
hepatoma cell lines by trichostatin A	30.37	2.03E-03
Upregulated by butyrate in SW260 colon carcinoma cells	10.83	2.84E-03
Enriched in mouse embryonic stem cells, compared to differentiated brain and bone marrow cells	3.07	8.89E-03

p values shown are after Bonferroni correction

RIBOSOME



Large subunit (Haloarcula marismortui)



Small subunit (Thermus aquaticus)

Ribosomal RNAs

Bacteria / Archaea	23S	5S		16S	
Eukaryotes	25S	5S	5.8S	18S	

Ribosomal proteins

B B/A A/E E	EF-Tu	S10 S20e	L3 L3e	L4 L4e	L23 L23Ae	L2 L8e	S19 S15e	L22 L17e	S3 S3e	RP-L16 L10e	L29 L35e	L7/L12 stalk													
B/A A/E E	S17 S11e	L14 L23e	L24 L26e	S4e	L5 L11e	S14 S29e	S8 S15Ae	L6 L9e	L32e	L19e	L18 L5e	S5 S2e	L30 L7e	L15 L27Ae	SecY										
B B/A A/E E	L34e	L14e	IF1	L36	S13 S18e	S11 S14e	S4 S9e	RpoA	L18e	L17	L13 L13Ae	S9 S16e													
B B/A A/E E	EF-Tu,G	S7 S5e	S12 S23e	L30e	L7A L7Ae	RpoC,B	L7/L12 A E	L12 LP1,LP2	L10 LP0	L1 L10Ae	L11 L12e														
B B/A A/E E	S2 SAe	EF-Ts	IF2	S15 S13e	IF3	L35	L20	L34	RF1	L31	L32	L9	S18	S6											
B	L28	L33	L21	L27	FtsY,Ffh	S16	L19	S1	S20	S21	S22	L25													
A/E	L10e	L13e	L15e	L21e	L24e	L31e	L35Ae	L37e	L37Ae	L39e	L40e	L41e	L44e												
A/E	S3Ae	S6e	S8e	S17e	S19e	S24e	S25e	S26e	S27e	S27Ae	S28e	S30e	A	LX											
E	L6e	L18Ae	L22e	L27e	L28e	L29e	L36e	L38e																	
E	S7e	S10e	S12e	S21e																					

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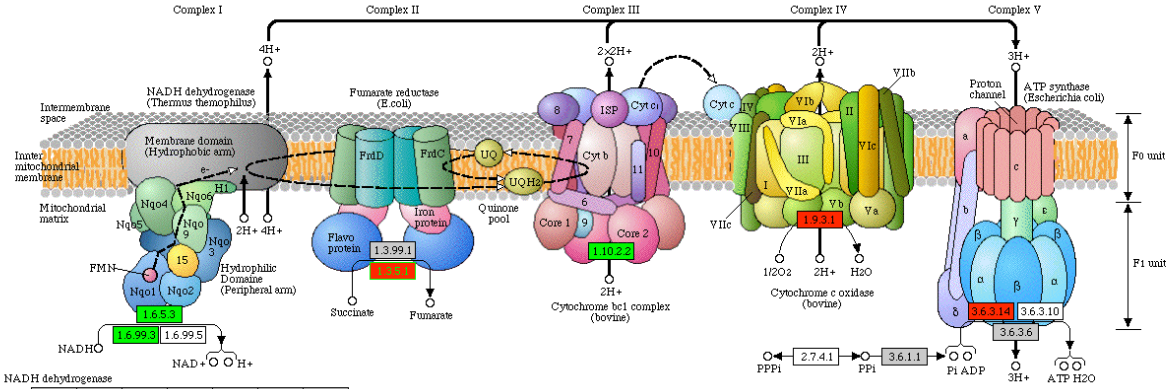
OXIDATIVE PHOSPHORYLATION



NADH dehydrogenase

E	ND1	ND2	ND3	ND4	ND4L	ND5	ND6															
E	Ndufs1	Ndufs2	Ndufs3	Ndufs4	Ndufs5	Ndufs6	Ndufs7	Ndufs8	Ndufv1	Ndufv2	Ndufv3											
B/A	NuoA	NuoB	NuoC	NuoD	NuoE	NuoF	NuoG	NuoH	NuoI	NuoJ	NuoK	NuoL	NuoM	NuoN								
B/A	NdhC	NdhK	NdhJ	NdhH	NdhA	NdhI	NdhG	NdhE	NdhF	NdhD	NdhB	NdhL	NdhM	NdhN	HoxE	HoxF	HoxU					
E	Ndufa1	Ndufa2	Ndufa3	Ndufa4	Ndufa5	Ndufa6	Ndufa7	Ndufa8	Ndufa9	Ndufa10	Ndufab1	Ndufa11										
E	Ndufb1	Ndufb2	Ndufb3	Ndufb4	Ndufb5	Ndufb6	Ndufb7	Ndufb8	Ndufb9	Ndufb10	Ndufc1	Ndufc2										

F-type ATPase (Bacteria)

beta	alpha	gamma	delta	epsilon	c	a	b

F-type ATPase (Eukaryotes)

beta	alpha	gamma	OSCP	delta	epsilon	c	a
b	e	f6	f	8			
d	f	h	j	k	g		

F-type ATPase (Prokaryotes)

A	B	C	D	E	F	I	K

F-type ATPase (Eukaryotes)

A	B	C	D	E	F	G	H
I	AC39	54kD	S1	lipid			

Succinate dehydrogenase / Fumarate reductase

E	SDHC	SDHD	SDHA	SDHB
B/A	SdhC	SdhD	SdhA	SdhB
			FrdA	FrdB	FrdC	FrdD

Cytochrome c reductase

E/B/A	ISP	Cyt b	Cyt 1					
E				COR1	QCR2	QCR6	QCR7	QCR8	QCR9	QCR10

Cytochrome c oxidase

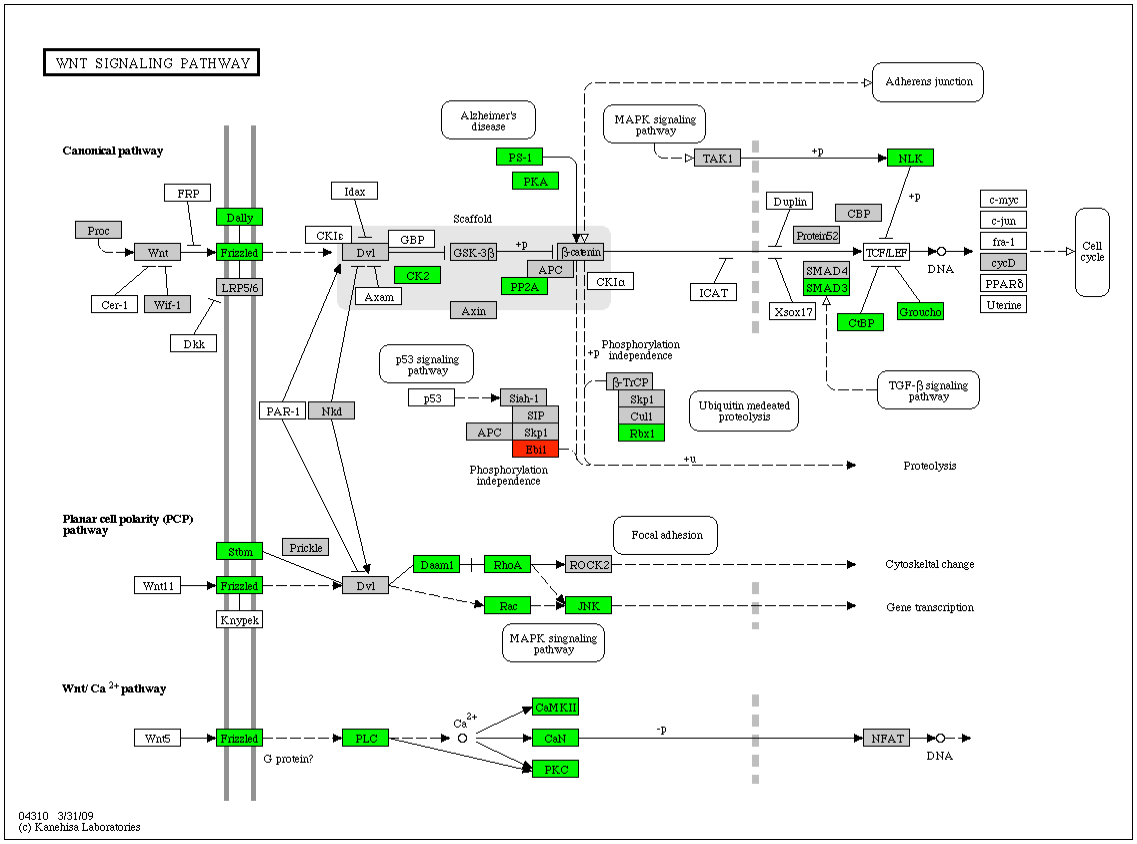
E	COX10		COX3	COX1	COX2	COX4	COX5A	COX5B	COX6A	COX6B	COX6C	COX7A	COX7B	COX7C	COX8	E/B/A		COX17
																COX11	COX15	
B/A	CyoE	CyoD	CyoC	CyoB	CyoA													
		CoxD	CoxC	CoxA	CoxB													
		QoxD	QoxC	QoxB	QoxA													

Cytochrome c oxidase , cbb3-type

B	I	II	IV	III

Cytochrome bd complex

B/A	CydA	CydB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Planar cell polarity (PCP) pathway
Wnt/ Ca 2+ pathway
Adherens junction
Alzheimer's disease
MAPK signaling pathway
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NLK
FRP
Idax
Dally
Scaffold
Duplin
CBP
Proc
CKIε
GBP
Protein62
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c-jun
fra-1
cycD
PPARδ
Uterine
Cell cycle
Wnt
Frizzled
Dvl
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APC
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PP2A
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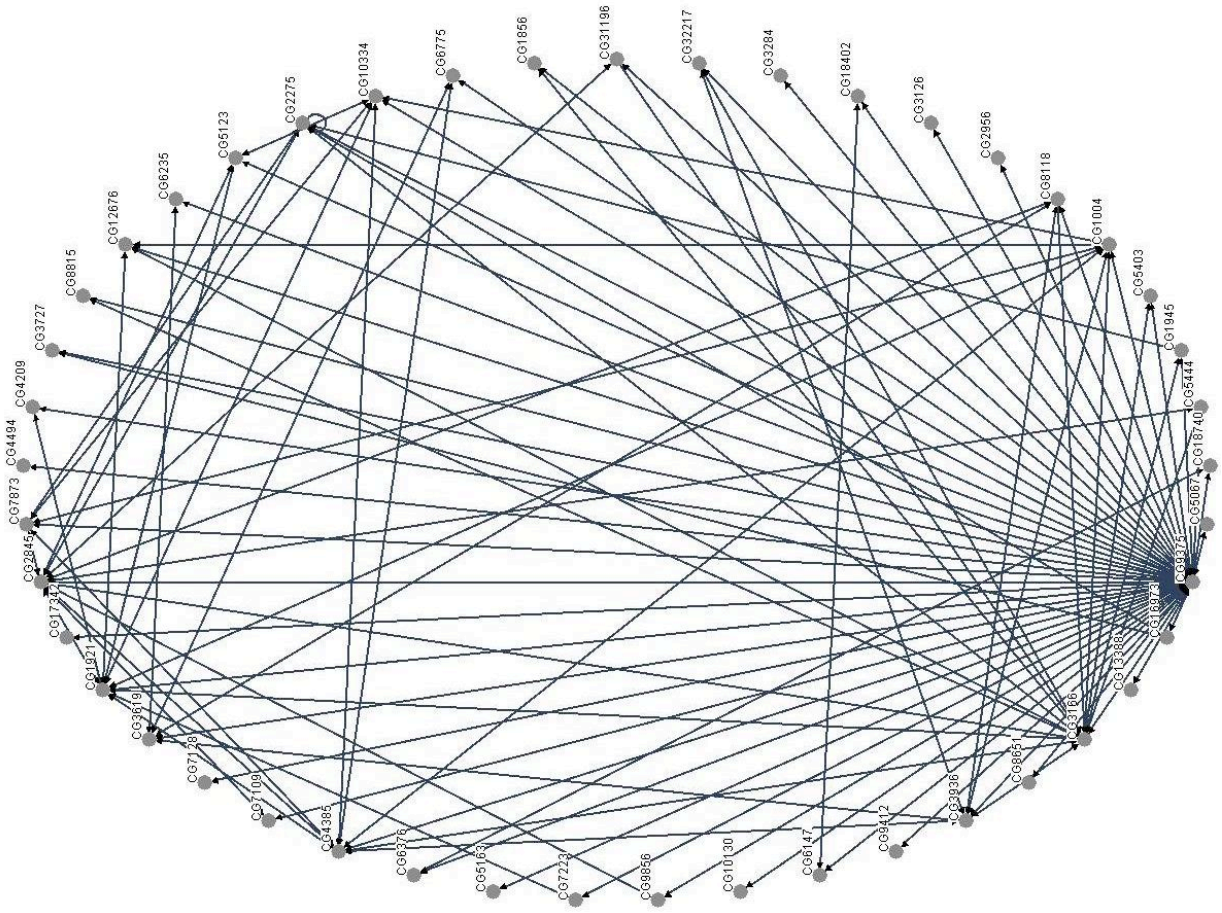
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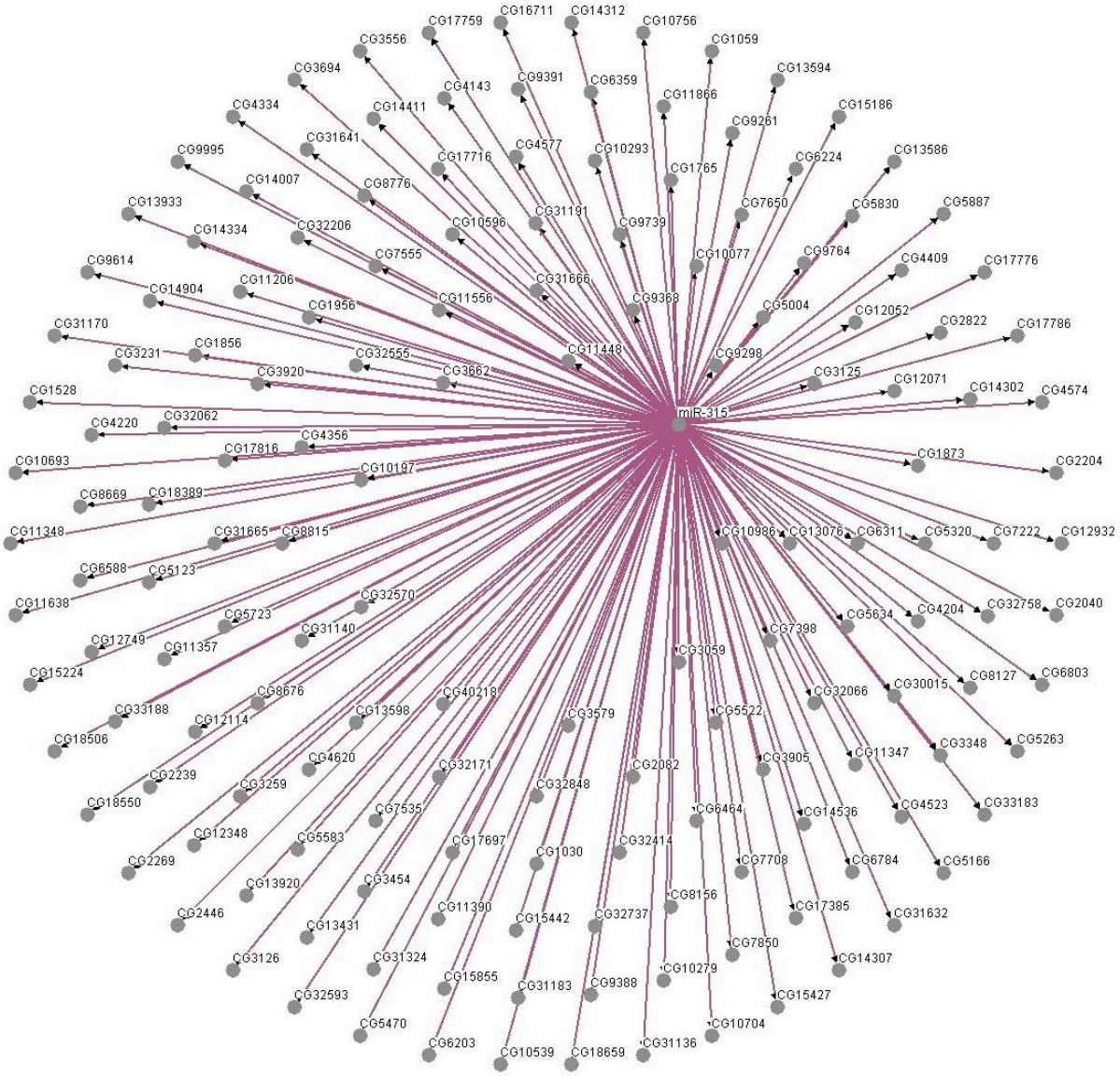
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