Published in final edited form as:

Mitochondrion. 2011 November ; 11(6): 994–995. doi:10.1016/j.mito.2011.08.014.

Mitochondrial Small RNAs that are Up-Regulated in Hippocampus during Olfactory Discrimination Training in Mice

Neil R. Smalheiser^{1,*}, Giovanni Lugli¹, Jyothi Thimmapuram², Edwin H. Cook¹, and John Larson¹

¹Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60612 USA

²W. M. Keck Center for Comparative and Functional Genomics, University of Illinois at Urbana-Champaign, Urbana, IL 61801

Abstract

Adult mice were trained to execute a nose-poke in a port containing one of two simultaneously present odors in order to obtain a reward. Hippocampus RNA of trained mice vs. controls was subjected to Illumina deep sequencing. Two mitochondrial RNAs (a tRNA and Mt-1) gave rise to 25–30-nt. small RNAs that showed a dramatic and specific increase with training (>50-fold relative to controls). Mt-1 is encoded within the termination association sequence (TAS) of the mitochondrial DNA control region. Small RNAs may link behavioral plasticity to protein synthesis and replication of mitochondria to support dendritic growth, spine stabilization, and synapse formation.

Keywords

microRNAs; RNA interference; synaptic plasticity; Mt-1; mitochondrial replication

Dear Sirs

In a recent study, we trained mice to learn an olfactory discrimination task, a hippocampus-dependent learning task, and carried out Illumina deep sequencing of 18–30 nt. hippocampus RNA (Smalheiser et al., 2011). Several classes of putative endogenous siRNAs were detected. As well, a novel class of small RNAs was detected that showed extremely large and specific changes with training -- these derive from abundant noncoding RNAs including ribosomal RNAs, snoRNAs, Y RNA and RMRP RNA, particularly at their 5' or 3' ends, at sites predicted to form intramolecular double-stranded RNA regions. Whereas these RNAs also gave rise constitutively to 20–23 nt. small RNAs, it was striking that longer versions of small RNAs (25–30 nt in length) arose from the same sites but were selectively expressed in the trained group, with little or no expression in the two control groups.

© 2011 Elsevier B.V. and Mitochondria Research Society. All rights reserved.

*Corresponding author: UIC Psychiatric Institute MC912, 1601 W. Taylor St., Chicago, IL 60612 USA, phone: 312-413-4581, fax: 312-413-4569, neils@uic.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Since changes in mitochondrial metabolism and gene expression accompany and foster synaptic plasticity (Williams et al, 1998; Li et al, 2004), we have now extended the analysis of the small RNAs from this dataset to include those that align to the mitochondrial genome. 124 unique sequences were well-expressed (e.g., >20 counts summed across all six pooled samples) and aligned to many, though not all, annotated mitochondrial transcripts. However, only two genomic loci gave rise to small RNAs that showed very large and specific up-regulation with training (Table 1). The first set of small RNAs aligned to the 5' end of a mitochondrial tRNA. Small RNAs arising from the 5' end of tRNAs are still poorly understood (Pederson, 2010), but at least some are thought to be processed by dicer and to associate with Argonaute proteins, where they can potentially target other RNA sequences (Cole et al., 2009).

The second set aligned to the 5' end of Mt-1, a 68 bp mouse-specific noncoding RNA transcript previously identified via deep sequencing by Lung et al. (2006) which exhibits variable length due to polyadenylation. This region is annotated as expressing an Ensembl transcript (ENSMUST 00000103243) that encodes a microRNA-like small RNA (mmu-mir-805). Mmu-mir-805 is highly expressed in isolated mitochondria (Bian et al., 2010) and is down-regulated in renal ischemic reperfusion injury (Godwin et al., 2010). It should be noted that shorter RNAs (< 24 nt) were also expressed at both loci, yet the large up-regulation with training was only observed for the size class of 25–30 nt RNAs (Table 1; discussed in Smalheiser et al, 2011). Mt-1/mmu-mir-805 is predicted to form a small hairpin microRNA precursor-like stem-loop structure, but the biogenesis of small RNAs from this locus is not clear: On the one hand, the 25–28 nt. small RNAs extend over the loop region, which is not a typical pattern seen in dicer-dependent formation of microRNAs (discussed in Chiang et al., 2009). On the other hand, some small RNAs that are derived from snoRNAs also extend over a loop in similar fashion and yet appear to be processed in a dicer-dependent manner (Ender et al, 2008).

Mt-1 is particularly intriguing, because this transcript is located in the termination acceptor sequence region of the mitochondrial DNA control region that is involved in DNA replication. This finding reawakens new interest in finding out the normal role(s) for the Mt-1 transcript and the various small RNAs that it encodes. Although Mt-1 is mouse-specific, it will be interesting to learn whether the mitochondrial DNA control region in other species also gives rise to small RNAs and whether they have regulatory functions in mitochondrial replication.

Acknowledgments

We thank Angela L. Lenon for assistance in training mice. Work was supported by the Jean Young and Walden W. Shaw Foundation, NIH (Autism Center of Excellence P50 HD055751), and the Stanley Medical Research Institute. Funding sources played no part in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the paper for publication.

References

- Bian Z, Li LM, Tang R, Hou DX, Chen X, Zhang CY, Zen K. Identification of mouse liver mitochondria-associated miRNAs and their potential biological functions. *Cell Res.* 2010; 20:1076–1078. [PubMed: 20733615]
- Chiang HR, Schoenfeld LW, Ruby JG, Auyeung VC, Spies N, Baek D, Johnston WK, Russ C, Luo S, Babiarz JE, Blelloch R, Schroth GP, Nusbaum C, Bartel DP. Mammalian microRNAs: experimental evaluation of novel and previously annotated genes. *Genes Dev.* 2010; 24:992–1009. [PubMed: 20413612]

- Cole C, Sobala A, Lu C, Thatcher SR, Bowman A, Brown JW, Green PJ, Barton GJ, Hutvagner G. Filtering of deep sequencing data reveals the existence of abundant Dicer-dependent small RNAs derived from tRNAs. *RNA*. 2009; 15:2147–2160. [PubMed: 19850906]
- Ender C, Krek A, Friedländer MR, Beitzinger M, Weinmann L, Chen W, Pfeffer S, Rajewsky N, Meister G. A human snoRNA with microRNA-like functions. *Mol Cell*. 2008; 32:519–528. [PubMed: 19026782]
- Godwin JG, Ge X, Stephan K, Jurisch A, Tullius SG, Iacomini J. Identification of a microRNA signature of renal ischemia reperfusion injury. *Proc Natl Acad Sci U S A*. 2010; 107:14339–14344. [PubMed: 20651252]
- Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*. 2004; 119:873–887. [PubMed: 15607982]
- Lung B, Zemann A, Madej MJ, Schuelke M, Techritz S, Ruf S, Bock R, Hüttenhofer A. Identification of small non-coding RNAs from mitochondria and chloroplasts. *Nucleic Acids Res*. 2006; 34:3842–3852. [PubMed: 16899451]
- Pederson T. Regulatory RNAs derived from transfer RNA? *RNA*. 2010; 16:1865–1869. [PubMed: 20719919]
- Smalheiser NR, Lugli G, Lenon AL, Davis JM, Torvik VI, Larson JR. Olfactory discrimination training up-regulates and reorganizes expression of microRNAs in adult mouse hippocampus. *ASN Neuro*. 2010; 2:art:e00028.
- Smalheiser NR, Lugli G, Thimmapuram J, Cook EH, Larson J. Endogenous siRNAs and noncoding RNA-derived small RNAs are expressed in adult mouse hippocampus and are up-regulated in olfactory discrimination training. *RNA*. 2011; 17:166–181. [PubMed: 21045079]
- Williams JM, Thompson VL, Mason-Parker SE, Abraham WC, Tate WP. Synaptic activity-dependent modulation of mitochondrial gene expression in the rat hippocampus. *Brain Res Mol Brain Res*. 1998; 60:50–56. [PubMed: 9748499]

Table 1

Mitochondrial loci giving rise to small RNAs up-regulated with training.

Seq	Nt	Seq start	Seq end	Nose-Poke A	Nose-Poke B	Pseudo-Train A	Pseudo-Train B	Training A	Training B
Aligning to a tRNA									
TAGGATAAGGTGTTTAGGTAG	21	3842	3822	21	23	35	32	13	23
TAGGATAAGGTGTTTAGGTAGC	22	3842	3821	46	46	38	55	27	60
TAGGATAAGGTGTTTAGGTAGCA	23	3842	3820	24	33	33	34	31	39
TAGGATAAGGTGTTTAGGTAGCAC	24	3842	3819	9	10	4	8	14	9
TAGGATAAGGTGTTTAGGTAGCAG	25	3842	3818	11	4	2	13	102	82
TAGGATAAGGTGTTTAGGTAGCACGG	26	3842	3817	10	5	3	13	1183	837
TAGGATAAGGTGTTTAGGTAGCACGGA	27	3842	3816	2		1	1	481	309
TAGGATAAGGTGTTTAGGTAGCACGGAG	28	3842	3815					70	34
TAGGATAAGGTGTTTAGGTAGCACGGAGA	29	3842	3814			1		29	9
Aligning to Mt-1 transcript									
GAATTGATCAGGACATA	17	16188	16172	388	328	223	263	57	283
GAATTGATCAGGACATAGGGT	21	16188	16168	445	354	348	434	375	369
GAATTGATCAGGACATAGGGTT	22	16188	16167	180	143	138	155	119	157
GAATTGATCAGGACATAGGGTTT	23	16188	16166	97	77	53	53	39	56
GAATTGATCAGGACATAGGGTTTG	24	16188	16165	38	41	6	37	67	78
GAATTGATCAGGACATAGGGTTTGA	25	16188	16164	27	25	2	23	163	248
GAATTGATCAGGACATAGGGTTTGAT	26	16188	16163	1			2	102	154
GAATTGATCAGGACATAGGGTTTGATA	27	16188	16162	2				946	669
GAATTGATCAGGACATAGGGTTTGATAG	28	16188	16161	1	1	2		261	143

Shown are the sequences that mapped to these loci; their length; their starting and ending positions on the mouse mitochondrial genome (all of the sequences aligned to the L strand); and the number of counts detected in each of the six pooled samples. "Training" samples were taken from mice who learned to discriminate two odors; "pseudo-train" were from yoked mice which underwent pseudo-training; and "nose-poke" were from yoked mice which performed nose-pokes but were not exposed to the odors. Each treatment group consisted of samples from 7 animals which were divided into 2 pooled samples A and B for deep sequencing (see Materials and methods in Smalheiser et al, 2010, 2011). Sequences that are 25 nt or longer are highlighted in yellow. The sequence corresponding to mmu-mir-805 is indicated in bold.