

Trypanosomatid mitochondrial RNA editing: Dramatically complex transcript repertoires revealed with a dedicated mapping tool

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Abstract

© The Author(s) 2017. RNA editing by targeted insertion and deletion of uridine is crucial to generate translatable mRNAs from the cryptogenes of the mitochondrial genome of kinetoplastids. This type of editing consists of a stepwise cascade of reactions generally proceeding from 3' to 5' on a transcript, resulting in a population of partially edited as well as pre-edited and completely edited molecules for each mitochondrial cryptogene of these protozoans. Often, the number of uridines inserted and deleted exceed the number of nucleotides that are genome-encoded. Thus, analysis of kinetoplastid mitochondrial transcriptomes has proven frustratingly complex. Here we present our analysis of *Leptomonas pyrrocoris* mitochondrial cDNA deep sequencing reads using T-Aligner, our new tool which allows comprehensive characterization of RNA editing, not relying on targeted transcript amplification and on prior knowledge of final edited products. T-Aligner implements a pipeline of read mapping, visualization of all editing states and their coverage, and assembly of canonical and alternative translatable mRNAs. We also assess T-Aligner functionality on a more challenging deep sequencing read input from *Trypanosoma cruzi*. The analysis reveals that transcripts of cryptogenes of both species undergo very complex editing that includes the formation of alternative open reading frames and whole categories of truncated editing products.

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References

- [1] Gray, M.W., Lukės, J., Archibald, J.M., Keeling, P.J. and Doolittle, W.F. (2010) Cell biology. Irremediable complexity? *Science*, 330, 920-921.
- [2] Koonin, E.V. (2016) Splendor and misery of adaptation, or the importance of neutral null for understanding evolution. *BMC Biol.*, 14, 114.
- [3] Lukeš, J., Archibald, J.M., Keeling, P.J., Doolittle, W.F. and Gray, M.W. (2011) How a neutral evolutionary ratchet can build cellular complexity. *IUBMB Life*, 63, 528-537
- [4] Stoltzfus, A. (2012) Constructive neutral evolution: exploring evolutionary theory's curious disconnect. *Biol Direct*, 7, 35.
- [5] Smith, D.R. and Keeling, P.J. (2016) Protists and the Wild, Wild West of gene expression: new frontiers, Lawlessness, and Misfits. *Annu. Rev. Microbiol.*, 70, 161-178.
- [6] Burger, G., Moreira, S. and Valach, M. (2016) Genes in Hiding. *Trends Genet.*, 32, 553-565.
- [7] Faktorová, D., Dobáková, E., Peña-Díaz, P. and Lukeš, J. (2016) From simple to supercomplex: mitochondrial genomes of euglenozoan protists. *F1000Research*, 5, 392.

- [8] Valach, M., Moreira, S., Faktorová, D., Lukeš, J. and Burger, G. (2016) Post-transcriptional mending of gene sequences: looking under the hood of mitochondrial gene expression in diplomonads. *RNA Biol.*, 13, 1204-1211.
- [9] Yabuki, A., Tanifuji, G., Kusaka, C., Takishita, K. and Fujikura, K. (2016) Hyper-eccentric structural genes in the mitochondrial genome of the algal parasite *Hemistasia phaeocysticola*. *Genome Biol. Evol.*, 8, 2870-2878.
- [10] Aphasizheva, I. and Aphasizhev, R. (2016) U-insertion/deletion mRNA-editing holoenzyme: definition in sight. *Trends Parasitol.*, 32, 144-156.
- [11] Read, L.K., Lukeš, J. and Hashimi, H. (2016) Trypanosome RNA editing: the complexity of getting U in and taking U out. *Wiley Interdiscip. Rev. RNA*, 7, 33-51.
- [12] David, V., Flegontov, P., Gerasimov, E., Tanifuji, G., Hashimi, H., Logacheva, M.D., Maruyama, S., Onodera, N.T., Gray, M.W., Archibald, J.M. et al. (2015) Gene loss and error-prone RNA editing in the mitochondrion of *Perkinsela*, an endosymbiotic kinetoplastid. *mBio.*, 6, e01498-15
- [13] Simpson, L., Thiemann, O.H., Savill, N.J., Alfonzo, J.D. and Maslov, D.A. (2000) Evolution of RNA editing in trypanosome mitochondria. *Proc. Natl. Acad. Sci. U.S.A.*, 97, 6986-6993.
- [14] Simpson, L. and Maslov, D.A. (1999) Evolution of the U-insertion/deletion RNA editing in mitochondria of kinetoplastid protozoa. *Ann. N. Y. Acad. Sci.*, 870, 190-205.
- [15] Aravin, A.A., Yurchenko, V., Merzlyak, E.M. and Kolesnikov, A.A. (1998) The mitochondrial ND8 gene from *Crithidia oncopelti* is not pan-edited. *FEBS Lett.*, 431, 457-460.
- [16] Gerasimov, E.S., Kostygov, A.Y., Yan, S. and Kolesnikov, A.A. (2012) From cryptogene to gene? ND8 editing domain reduction in insect trypanosomatids. *Eur. J. Protistol.*, 48, 185-193.
- [17] McDermott, S.M., Luo, J., Carnes, J., Ranish, J.A. and Stuart, K. (2016) The architecture of *Trypanosoma brucei* editosomes. *Proc. Natl. Acad. Sci. U.S.A.*, 113, e6476-e6485.
- [18] Simpson, R.M., Bruno, A.E., Chen, R., Lott, K., Tylec, B.L., Bard, J.E., Sun, Y., Buck, M.J. and Read, L.K. (2017) Trypanosome RNA Editing Mediator Complex proteins have distinct functions in gRNA utilization. *Nucleic Acids Res.*, 45, 7965-7983.
- [19] Kirby, L.E., Sun, Y., Judah, D., Nowak, S. and Koslowsky, D. (2016) Analysis of the *Trypanosoma brucei* EATRO 164 bloodstream guide RNA transcriptome. *PLoS Negl. Trop. Dis.*, 10, e0004793.
- [20] Koslowsky, D., Sun, Y., Hindenach, J., Theisen, T. and Lucas, J. (2014) The insect-phase gRNA transcriptome in *Trypanosoma brucei*. *Nucleic Acids Res.*, 42, 1873-1886.
- [21] Simpson, L., Douglass, S.M., Lake, J.A., Pellegrini, M. and Li, F. (2015) Comparison of the mitochondrial genomes and steady state transcriptomes of two strains of the trypanosomatid parasite, *Leishmania tarentolae*. *PLoS Negl. Trop. Dis.*, 9, e0003841.
- [22] Ammerman, M.L., Presnyak, V., Fisk, J.C., Foda, B.M. and Read, L.K. (2010) TbRGG2 facilitates kinetoplastid RNA editing initiation and progression past intrinsic pause sites. *RNA*, 16, 2239-2251.
- [23] Koslowsky, D.J., Bhat, G.J., Read, L.K. and Stuart, K. (1991) Cycles of progressive realignment of gRNA with mRNA in RNA editing. *Cell*, 67, 537-546.
- [24] Simpson, R.M., Bruno, A.E., Bard, J.E., Buck, M.J. and Read, L.K. (2016) High-throughput sequencing of partially edited trypanosome mRNAs reveals barriers to editing progression and evidence for alternative editing. *RNA*, 22, 677-695.
- [25] Arts, G.J., van der Spek, H., Speijer, D., van den Burg, J., van Steeg, H., Sloof, P. and Benne, R. (1993) Implications of novel guide RNA features for the mechanism of RNA editing in *Crithidia fasciculata*. *EMBO J.*, 12, 1523-1532.
- [26] Maslov, D.A. and Simpson, L. (1992) The polarity of editing within a multiple gRNA-mediated domain is due to formation of anchors for upstream gRNAs by downstream editing. *Cell*, 70, 459-467.
- [27] Maslov, D.A., Thiemann, O. and Simpson, L. (1994) Editing and misediting of transcripts of the kinetoplast maxicircle G5 (ND3) cryptogene in an old laboratory strain of *Leishmania tarentolae*. *Mol. Biochem. Parasitol.*, 68, 155-159.
- [28] Sturm, N.R., Maslov, D.A., Blum, B. and Simpson, L. (1992) Generation of unexpected editing patterns in *Leishmania tarentolae* mitochondrial mRNAs: misediting produced by misguiding. *Cell*, 70, 469-476.
- [29] Ochsenreiter, T. and Hajduk, S.L. (2006) Alternative editing of cytochrome c oxidase III mRNA in trypanosome mitochondria generates protein diversity. *EMBO Rep.*, 7, 1128-1133.
- [30] Ochsenreiter, T., Anderson, S., Wood, Z.A. and Hajduk, S.L. (2008) Alternative RNA editing produces a novel protein involved in mitochondrial DNA maintenance in trypanosomes. *Mol. Cell. Biol.*, 28, 5595-5604.
- [31] Ochsenreiter, T., Cipriano, M. and Hajduk, S.L. (2008) Alternative mRNA editing in trypanosomes is extensive and may contribute to mitochondrial protein diversity. *PLoS One.*, 3, e1566.
- [32] Madina, B.R., Kumar, V., Metz, R., Mooers, B.H., Bundschuh, R. and Cruz-Reyes, J. (2014) Native mitochondrial RNA-binding complexes in kinetoplastid RNA editing differ in guide RNA composition. *RNA*, 20, 1142-1152.
- [33] Flegontov, P., Gray, M.W., Burger, G. and Lukeš, J. (2011) Gene fragmentation: a key to mitochondrial genome evolution in Euglenozoa? *Curr. Genet.*, 57, 225-232.
- [34] Gray, M.W. (2012) Evolutionary origin of RNA editing. *Biochemistry*, 51, 5235-5242.

- [35] Speijer, D. (2006) Is kinetoplastid pan-editing the result of an evolutionary balancing act? *IUBMB Life*, 58, 91-96.
- [36] Speijer, D. (2011) Does constructive neutral evolution play an important role in the origin of cellular complexity? Making sense of the origins and uses of biological complexity. *Bioessays*, 33, 344-349.
- [37] Kirby, L.E. and Koslowsky, D. (2017) Mitochondrial dual-coding genes in *Trypanosoma brucei*. *PLoS Negl. Trop. Dis.*, 11, e0005989.
- [38] Maslov, D.A., Sturm, N.R., Niner, B.M., Gruszynski, E.S., Peris, M. and Simpson, L. (1992) An intergenic G-rich region in *Leishmania tarentolae* kinetoplast maxicircle DNA is a pan-edited cryptogene encoding ribosomal protein S12. *Mol. Cell. Biol.*, 12, 56-67.
- [39] Feagin, J.E., Abraham, J.M. and Stuart, K. (1988) Extensive editing of the cytochrome c oxidase III transcript in *Trypanosoma brucei*. *Cell*, 53, 413-422.
- [40] Shaw, J.M., Feagin, J.E., Stuart, K. and Simpson, L. (1988) Editing of kinetoplastid mitochondrial mRNAs by uridine addition and deletion generates conserved amino acid sequences and AUG initiation codons. *Cell*, 53, 401-411.
- [41] Feagin, J.E., Shaw, J.M., Simpson, L. and Stuart, K. (1988) Creation of AUG initiation codons by addition of uridines within cytochrome b transcripts of kinetoplastids. *Proc. Natl. Acad. Sci. U.S.A.*, 85, 539-543.
- [42] Carnes, J., McDermott, S., Anupama, A., Oliver, B.G., Sather, D.N. and Stuart, K. (2017) In vivo cleavage specificity of *Trypanosoma brucei* editosome endonucleases. *Nucleic Acids Res.*, 45, 4667-4686.
- [43] del Campo, J., Sieracki, M.E., Molestina, R., Keeling, P., Massana, R. and Ruiz-Trillo, I. (2014) The others: our biased perspective of eukaryotic genomes. *Trends Ecol. Evol.*, 29, 252-259.
- [44] Votýpka, J., Klepetková, H., Yurchenko, V.Y., Horák, A., Lukš, J. and Maslov, D.A. (2012) Cosmopolitan distribution of a trypanosomatid *Leptomonas pyrrocoris*. *Protist.*, 163, 616-631.
- [45] Maslov, D.A., Votýpka, J., Yurchenko, V. and Lukeš, J. (2013) Diversity and phylogeny of insect trypanosomatids: all that is hidden shall be revealed. *Trends Parasitol.*, 29, 43-52.
- [46] Flegontov, P., Butenko, A., Firsov, S., Kraeva, N., Eliáš, M., Field, M.C., Filatov, D., Flegontova, O., Gerasimov, E.S., Hlaváčová, J. et al. (2016) Genome of *Leptomonas pyrrocoris*: a high-quality reference for monoxenous trypanosomatids and new insights into evolution of *Leishmania*. *Sci. Rep.*, 6, 23704.
- [47] Castellani, O., Ribeiro, L.V. and Fernandes, J.F. (1967) Differentiation of *Trypanosoma cruzi* in culture. *J. Protozool.*, 14, 447-451.
- [48] Pelletier, M., Read, L.K. and Aphasizhev, R. (2007) Isolation of RNA binding proteins involved in insertion/deletion editing. *Methods Enzymol.*, 424, 75-105.
- [49] Horváth, A., Horáková, E., Dunajčíková, P., Verner, Z., Pravdová, E., Slapetová, I., Cuninková, L. and Lukes, J. (2005) Downregulation of the nuclear-encoded subunits of the complexes III and IV disrupts their respective complexes but not complex I in procyclic *Trypanosoma brucei*. *Mol. Microbiol.*, 58, 116-130.
- [50] Záhonová, K., Hadariová, L., Vacula, R., Yurchenko, V., Eliáš, M., Krajčovič, J. and Vesteg, M. (2014) A small portion of plastid transcripts is polyadenylated in the flagellate *Euglena gracilis*. *FEBS Lett.*, 588, 783-788.
- [51] Zhang, L., Sement, F.M., Suematsu, T., Yu, T., Monti, S., Huang, L., Aphasizhev, R. and Aphasizheva, I. (2017) PPR polyadenylation factor defines mitochondrial mRNA identity and stability in trypanosomes. *EMBO J.*, 36, 2435-2454.
- [52] Gazestani, V.H., Hampton, M., Shaw, A.K., Liggett, C., Salavati, R. and Zimmer, S.L. (2017) Tail characteristics of *Trypanosoma brucei* mitochondrial transcripts are developmentally altered in a transcript-specific manner. *Int. J. Parasitol.*, doi:10.1016/j.ijpara.2017.08.012.
- [53] Aslett, M., Aurrecochea, C., Berriman, M., Brestelli, J., Brunk, B.P., Carrington, M., Depledge, D.P., Fischer, S., Gajria, B., Gao, X. et al. (2010) TriTrypDB: a functional genomic resource for the Trypanosomatidae. *Nucleic Acids Res.*, 38, D457-D462.
- [54] Souza, A.E., Myler, P.J. and Stuart, K. (1992) Maxicircle CR1 transcripts of *Trypanosoma brucei* are edited and developmentally regulated and encode a putative iron-sulfur protein homologous to an NADH dehydrogenase subunit. *Mol. Cell. Biol.*, 12, 2100-2107.
- [55] Read, L.K., Myler, P.J. and Stuart, K. (1992) Extensive editing of both processed and preprocessed maxicircle CR6 transcripts in *Trypanosoma brucei*. *J. Biol. Chem.*, 267, 1123-1128.
- [56] Ruvalcaba-Trejo, L.I. and Sturm, N.R. (2011) The *Trypanosoma cruzi* Sylvio X10 strain maxicircle sequence: the third musketeer. *BMC Genomics*, 12, 58.
- [57] Langmead, B. and Salzberg, S. (2012) Fast gapped-read alignment with Bowtie 2. *Nat. Methods*, 9, 357-359.
- [58] Gazestani, V.H., Hampton, M., Abraham, J.E., Salavati, R. and Zimmer, S.L. (2016) circTAIL-seq, a targeted method for deep analysis of RNA 3' tails, reveals transcript-specific differences by multiple metrics. *RNA*, 22, 477-486.
- [59] Jirků, M., Yurchenko, V.Y., Lukes, J. and Maslov, D.A. (2012) New species of insect trypanosomatids from Costa Rica and the proposal for a new subfamily within the Trypanosomatidae. *J. Eukaryot. Microbiol.*, 59, 537-547.
- [60] Landweber, L.F. and Gilbert, W. (1993) RNA editing as a source of genetic variation. *Nature*, 363, 179-182.

- [61] Yasuhira, S. and Simpson, L. (1995) Minicircle-encoded guide RNAs from *Crithidia fasciculata*. *RNA*, 1, 634-643
- [62] Neboháčová, M., Kim, C.E., Simpson, L. and Maslov, D.A. (2009) RNA editing and mitochondrial activity in promastigotes and amastigotes of *Leishmania donovani*. *Int. J. Parasitol*, 39, 635-644.
- [63] Read, L.K., Wilson, K.D., Myler, P.J. and Stuart, K. (1994) Editing of *Trypanosoma brucei* maxicircle CR5 mRNA generates variable carboxy terminal predicted protein sequences. *Nucleic Acids Res.*, 22, 1489-1495.
- [64] Maslov, D.A. (2010) Complete set of mitochondrial pan-edited mRNAs in *Leishmania mexicana amazonensis* LV78. *Mol Biochem. Parasitol*, 173, 107-114.
- [65] Westenberger, S.J., Cerqueira, G.C, El-Sayed, N.M., Zingales, B., Campbell, D.A. and Sturm, N.R. (2006) *Trypanosoma cruzi* mitochondrial maxicircles display species- and strain-specific variation and a conserved element in the non-coding region. *BMC Genomics*, 7, 60.
- [66] Thomas, S., Martinez, L.L., Westenberger, S.J. and Sturm, N.R. (2007) A population study of the minicircles in *Trypanosoma cruzi*: predicting guide RNAs in the absence of empirical RNA editing. *BMC Genomics*, 8, 133.
- [67] Greif, G., Rodriguez, M., Reyna-Bello, A., Robello, C. and Alvarez-Valin, F. (2015) Kinetoplast adaptations in American strains from *Trypanosoma vivax*. *Mutat. Res.*, 773, 69-82.
- [68] Etheridge, R.D., Aphasizheva, I., Gershon, P.D. and Aphasizhev, R. (2008) 3' adenylation determines mRNA abundance and monitors completion of RNA editing in *T. brucei* mitochondria. *EMBO J.*, 27, 1596-1608.
- [69] Flegontova, O., Flegontov, P., Malviya, S., Audic, S., Wincker, P., de Vargas, C, Bowler, C., Lukes, J. and Horák, A. (2016) Extreme diversity of diplomonid eukaryotes in the ocean. *Curr. Biol*, 26, 3060-3065.
- [70] Gawryluk, R.M., Del Campo, J., Okamoto, N, Strassert, J.F., Lukes, J., Richards, T.A., Worden, A.Z., Santoro, A.E. and Keeling, P.J. (2016) Morphological identification and single-cell genomics of marine diplomonids. *Curr. Biol*, 26, 3053-3059.
- [71] de Vargas, C, Audic, S., Henry, N, Decelle, J., Mahe, F., Logares, R., Lara, E., Berney, C, Le Bescot, N, Probert, I. et al (2015) Ocean plankton. Eukaryotic plankton diversity in the sunlit ocean. *Science*, 348, 1261605.
- [72] Lukes, J., Skalicky, T., Tyč, J., Votypka, J. and Yurchenko, V. (2014) Evolution of parasitism in kinetoplastid flagellates. *Mol Biochem. Parasitol*, 195, 115-122.
- [73] Rodrigues, J.C., Godinho, J.L. and de Souza, W. (2014) Biology of human pathogenic trypanosomatids: epidemiology, lifecycle and ultrastructure. *Subcell Biochem.*, 74, 1-42.
- [74] Parsons, M., Ramasamy, G, Vasconcelos, E.J., Jensen, B.C. and Myler, P.J. (2015) Advancing *Trypanosoma brucei* genome annotation through ribosome profiling and spliced leader mapping. *Mol Biochem. Parasitol*, 202, 1-10.