

Evolution of mitochondrial rRNAs

Gertraud Burger



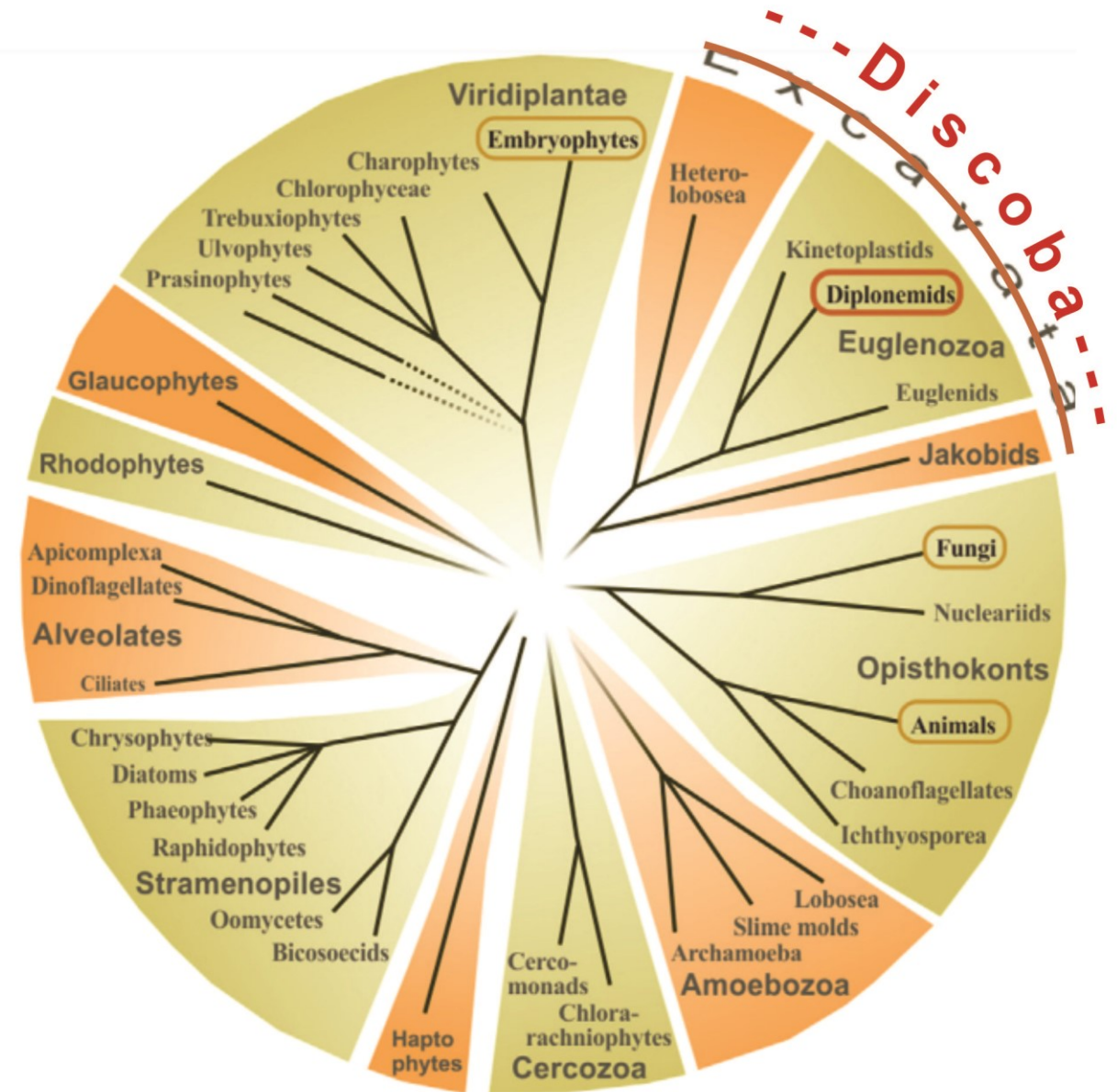
Montreal, QC, Canada

gertraud.burger@gmail.com

Computational Approaches to RNA Structure and Function
(Benasque Science Center, Jul 21 - Aug 03, 2024)

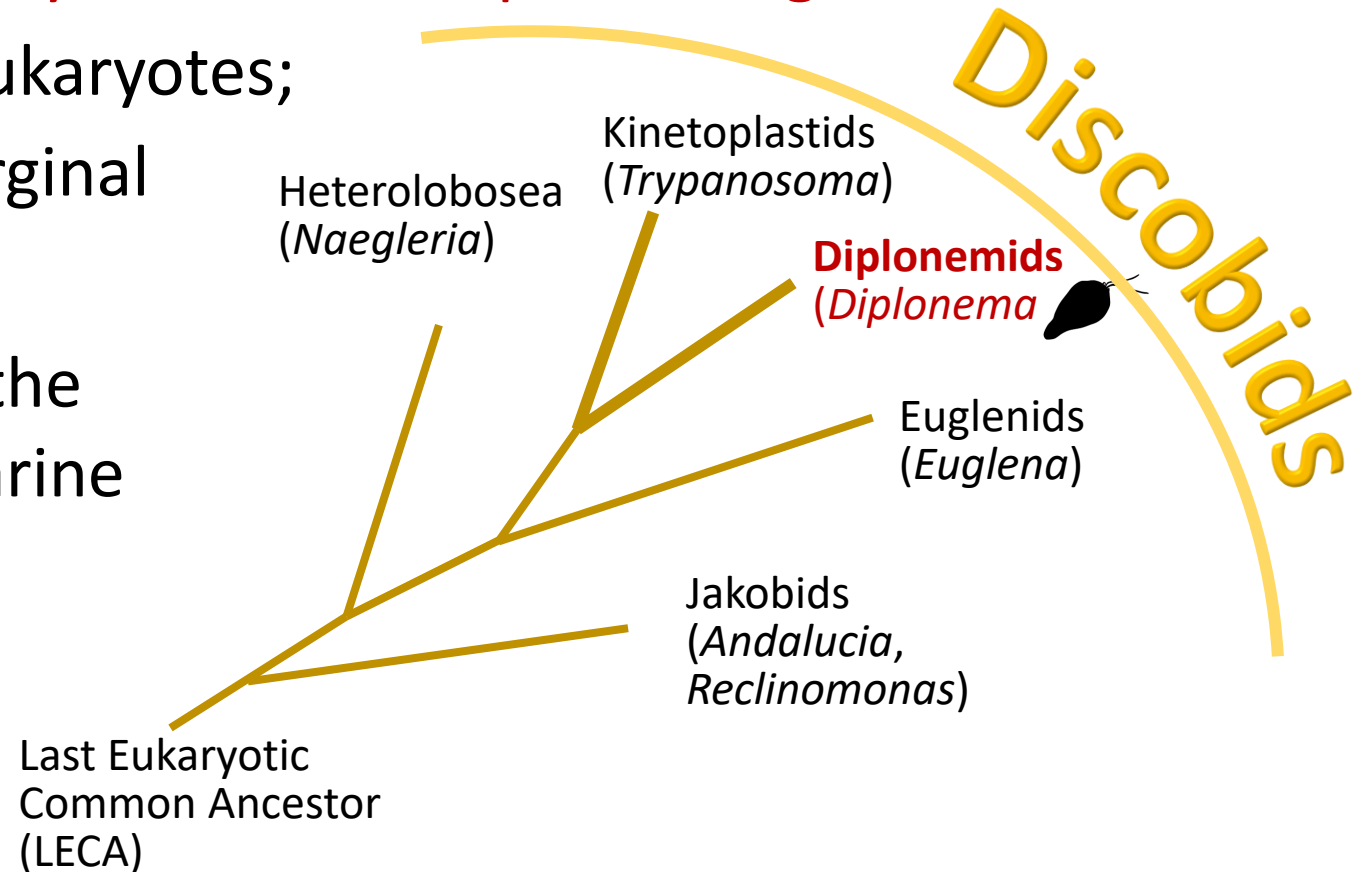
Mt rRNA evolution in discobids

- Discobids: one of the ~10 eukaryotic supergroups (best-known supergroups are Plantae and {Animals/Fungi});
- Include very basally diverging, minimally derived (jakobids – most bacteria-like mt genome), moderately, and rapidly evolving taxa;
- Therefore, ideal for evolutionary studies.



The discobid group **diplonemids**

- ‘New Kid on the discobid Block’ is diplomonids;
- Studied in our lab for their highly unusual RNA processing¹;
- Marine heterotrophic micro-eukaryotes;
- Traditionally considered of marginal ecological importance;
- Recently recognized as one of the most diverse and abundant marine eukaryotic groups²;
- Least distant model organisms: trypanosomes.

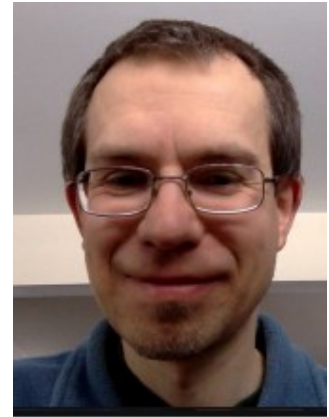


(1) Burger G et al (2016) Trends Genet 2:535; (2) de Vargas et al (2015) Science 348

Reminder

- Translation apparatus of mitochondria originates from that of their alpha-proteobacterial ancestor;
- Is distinct from the cytosolic system;
- RNA components: mt SSU rRNA, mt LSU rRNA, [sometimes mt 5S rRNA].

What follows: our work on mt rRNA in diplomonids; structures modeled manually with the help of phylogeny-based consensus models¹, RNAfold², and some covariance analysis.



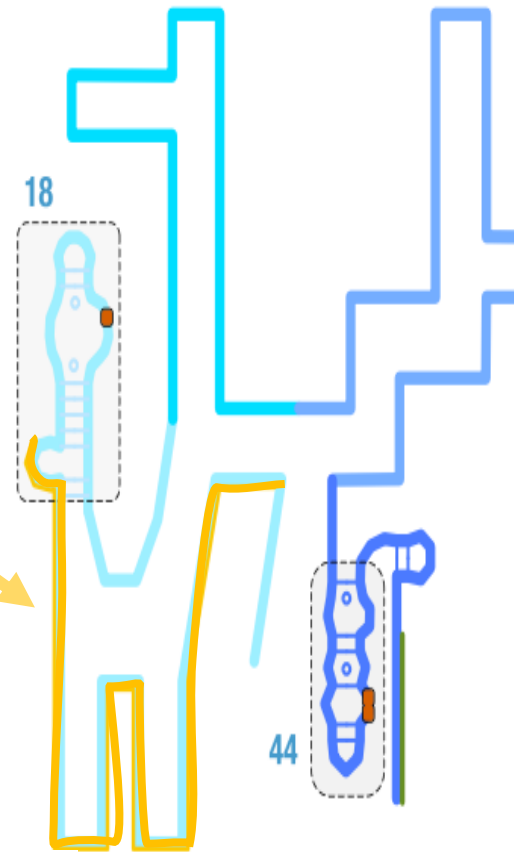
(1) Comparative RNA Web (<http://www.rna.cccb.utexas.edu>); Cannone et al (2002) BMC Bioinfo 3:2;

(2) Gruber et al (2008) Nucleic Acids Res 36:W70

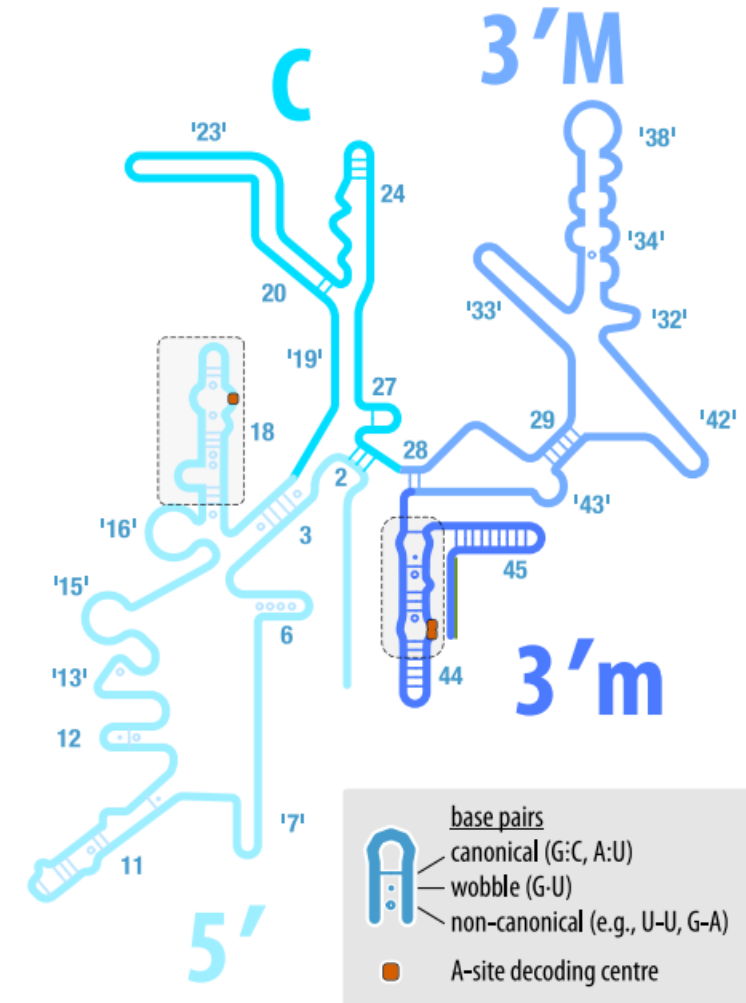
The mt **SSU**-rRNA in diplomonids

- **Shortest** molecule ever observed (~ 380 nt; ~25% of bacterial, 40% of trypanosome SSU rRNA);
- Only H18 and H44 readily recognizable (decoding center);
- ~ 40 **inosine** residues in 5' end (only case of I-containing rRNA);
- *Diplonema* model inspired by the *Trypanosoma* rRNA (cryoEM).

Diplonema papillatum
(covariance-based model)



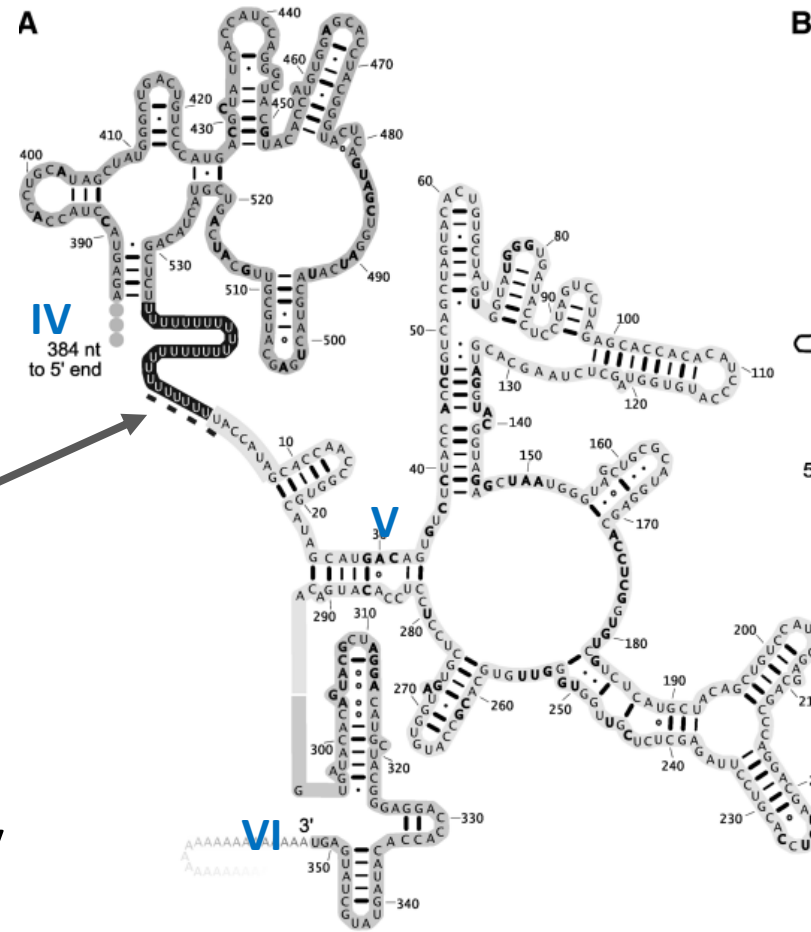
A *Trypanosoma brucei*
(cryo-EM-based model)



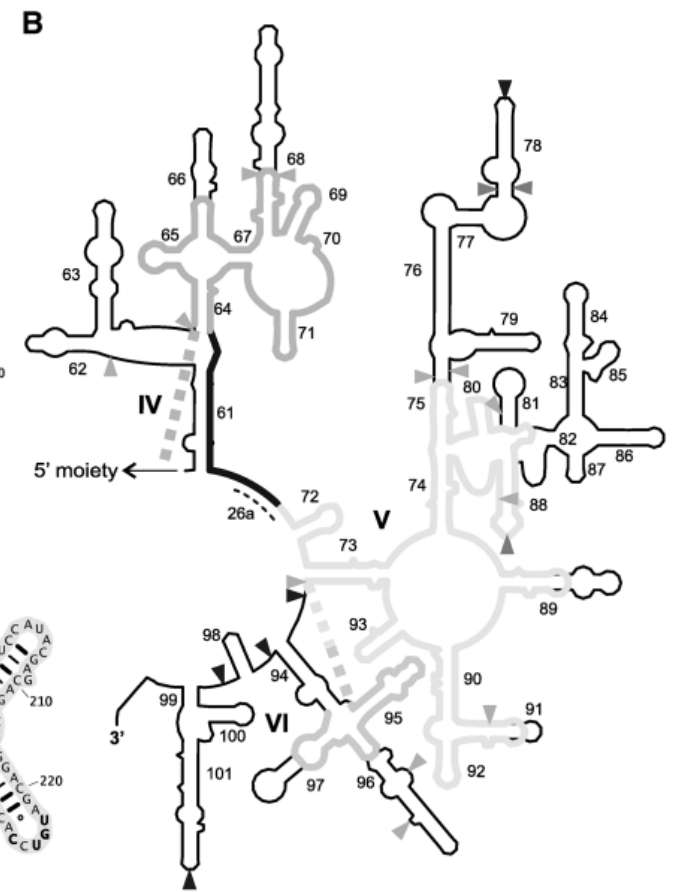
Valach M et al (2023) Nucleic Acids Res 42(4):2660-2672

The mt LSU-rRNA in diplonemids

- Among the **shortest** observed (~ 910 nt, = ~25% of bacterial LSU rRNA);
- The **3' half** has conserved elements, but not so the 5' portion;
- **Tract of ~ 30 Us** in domain IV;
- No notable structural similarity to the *Trypanosoma* rRNA.



Structure model for *D. papillatum*



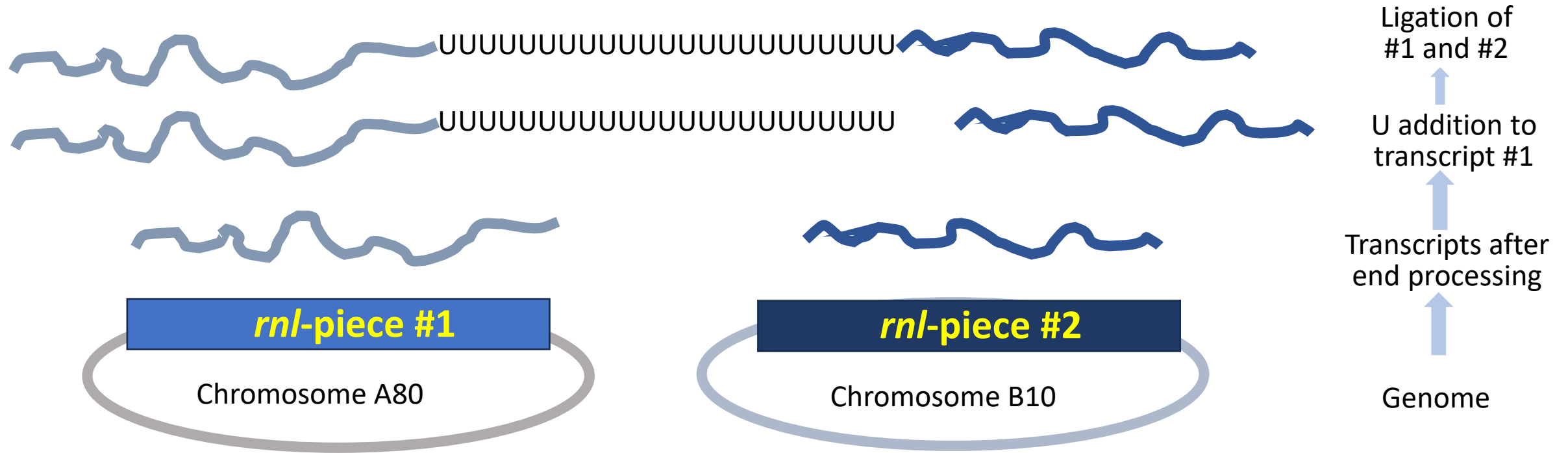
Superimposed on *E. coli* structure (black lines: elements **absent** from *D.p.*)

Valach M et al (2014) Nucleic Acids Res 51:6443-6460



Side note: Unusual rRNA features arise through editing

- **U tract** in *Diplonema's* mt LSU-rRNA arises through U appendage and RNA ligation





Side note _{ctnd}: Unusual rRNA features arise through editing

- **Inosines** in *Diplonema's* mt SSU-rRNAs occur by post-transcriptional A-to-I deamination (experimentally demonstrated; the ~100-nt long regions also undergoes massive C-to-U RNA editing)

59–143 **C**CC**A**GTGGGT**A**TGTGCTCTGT**A**CCGCTGT**A**CGGT**A**CT**A**GT**A**TCCATCC**A**CGC**A**GT**A**CGTCC**A**CC**A**TGCTGTGCT**A**TC**A**GCCC
xxx+ x + x x +xx x +x +x + x + xx+ xx+x x+ x +x xx+xx+ x x + x+ xxx
TTT**G**TTGGGT**G**TGTGTTTGT**G**TTGTTGT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT**G**TT

-> ‘Detect’ inosines in transcripts via searching A/G differences in sequence comparison of genome and transcriptome (instead of painstaking experimental screening).

The diplonemid mito-ribosome



- Is mito-ribosome as reduced as are mt-rRNAs?
- Recently established genetic transformation methodology¹ and nuclear genome sequence² of the type species *Diplonema papillatum* allowed analysis of the mt-ribosomal protein composition.


Miniature RNAs are embedded in an exceptionally protein-rich mitoribosome via an elaborate assembly pathway

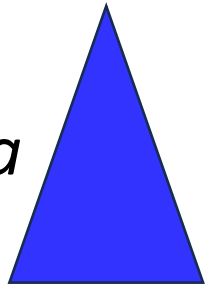
Matus Valach ^{1,*}, Corinna Benz ^{2,*}, Lisbeth C. Aguilar ³, Ondřej Gahura ²,
Drahomíra Faktorová ^{2,4}, Alena Zíková ^{2,4}, Marlene Oeffinger ^{1,3,5}, Gertraud Burger ¹,
Michael W. Gray ⁶ and Julius Lukeš ^{2,4}

Nucleic Acids Res (2023) 42:2260

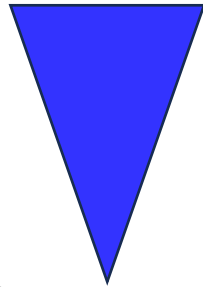
(1) Kaur B et al (2018) Environ Microbiol 20:1030-1040. (2) Valach M et al (2023) BMC Biol 21:99

Mt rRNAs & ribosomes across discobids

 *Diplonema*
Trypanosoma
Andalucia

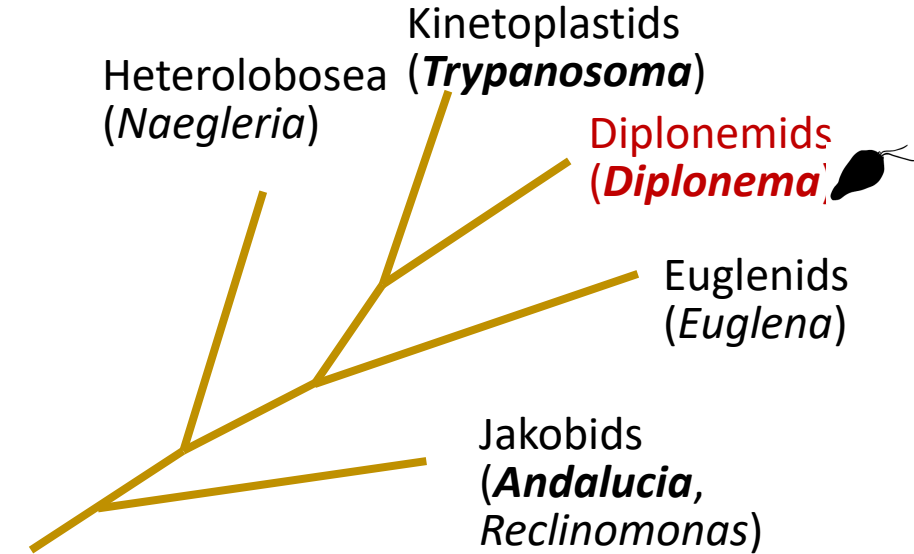


RNA size +
structural elements



Nr. of mt riboproteins

Numbers...



Discoban species	Size mt SSU-rRNA	Size mt LSU-rRNA	# of mt r-proteins	# of core proteins	# access. prots # shared with:
<i>Diplonema</i>	380 nt	910 nt	130	36/54	94 32 Tryp
<i>Trypanosoma</i> (1)	611 nt	1,150 nt	122	34/54	88 32 Dipl
<i>Andalucia</i> (2,3)	1,250 nt	2,570 nt	75	52/54	
Yeast	1,570 nt	3,290 nt	84	51/54	

(1) Ramrath et al 2018 Science 362; (2) Gray et al 2019 BMC Biol 18:22; (3) Burger et 2013 Genome Biol Evol 5:418

Speculations



- The extra mitoribosomal proteins in diplomonids replace missing rRNA structural elements;
- The numerous, clustered inosines and the long U tract in mt rRNAs cause
 - less rigid structures
 - more alternative structures;
- The extra mitoribosomal proteins in diplomonids also assure that rRNAs assume their functional structure.

To test these ideas ...

Next steps



Experimental

- Resolve *Diplonema* mt rRNA & mitoribosome structure by CryoEM [Naschberger (KAUST, SA) & Tobiasson (NCBI)];
- Identify & map additional **mt-rRNA modifications** by RNA mass spectrometry (R. Ross, Thermo Fisher Scientific).

In silico ! colaboraciones son bienvenidas !

- Now, sequences from 10 other diplomemids are available for **rigorous computational** analyses of the mt rRNAs, to
 - cope with the pronounced sequence divergence;
 - consider inosines in base pairing;
 - eventually compare CryoEM-derived and MFE rRNA structures.

Acknowledgements

Most of the presented work: Matus Valach, PhD, research assistant, my wet-lab

Collaborations (presented projects)

Michael W. Gray (Dalhousie University, Halifax, Canada)

B. Franz Lang (Université de Montréal, Canada)

Julius Lukeš (Parasitology Institute BC CAS, Budweis, Czech Republic)

Marlene Oeffinger (IRCM, Montreal, Canada; affinity purify & MS)

Andreas Naschberger (KAUST, Saudi-Arabia; CryoEM)

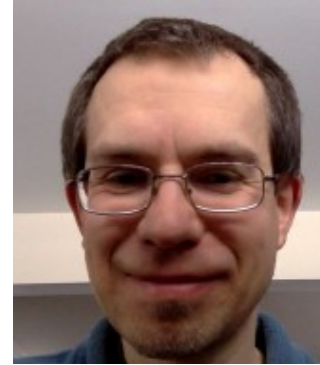
Victor Tobiasson (NCBI, USA; CryoEM-data analysis)

Robert Ross (Thermo Fisher Scientific, USA; RNA MS).

Current & previous lab members participating on the diplonemid projects:

Roxana Tarabuta, Alexandra Léveillé-Kunst, Felix A. LeSieur, Sandrine Moreira, Paul Stretenowich, Mohamed Aoulad Aissa, Georgette N. Kiethega, Yifei Yan, Sophie Breton, William Marande, Shona Teijeiro; & many internship students.

THE END



GenomeQuébec



CIHR IRSC

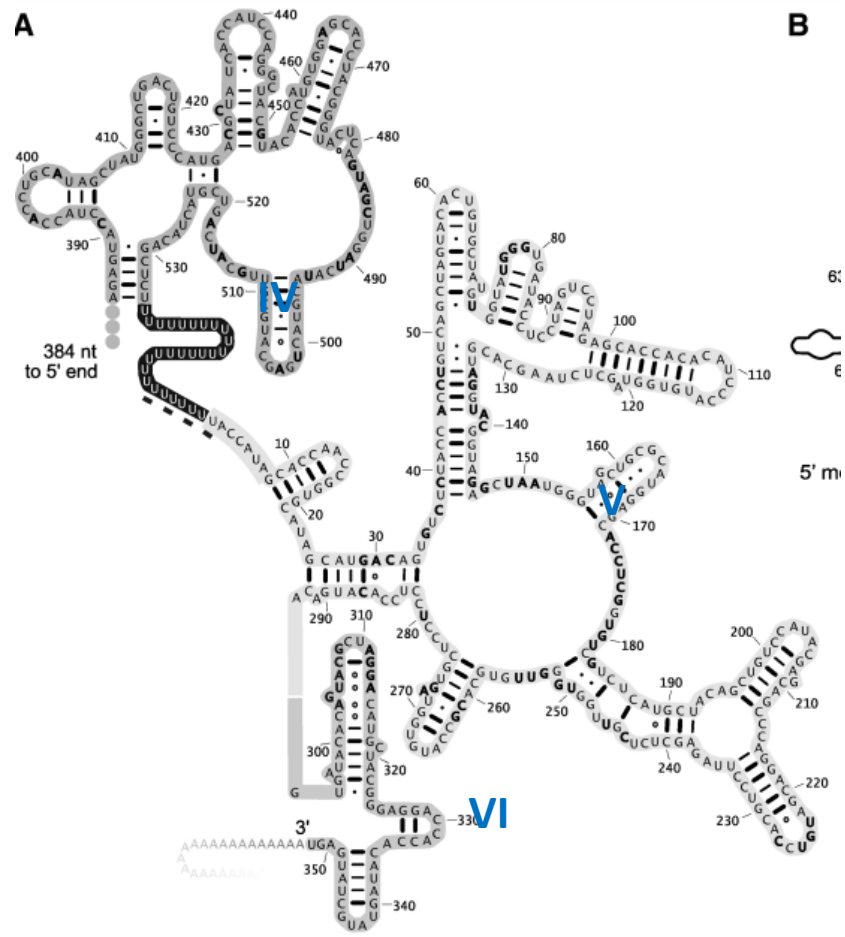
Canadian Institutes of Health Research Institut de recherche en santé publique

13/13

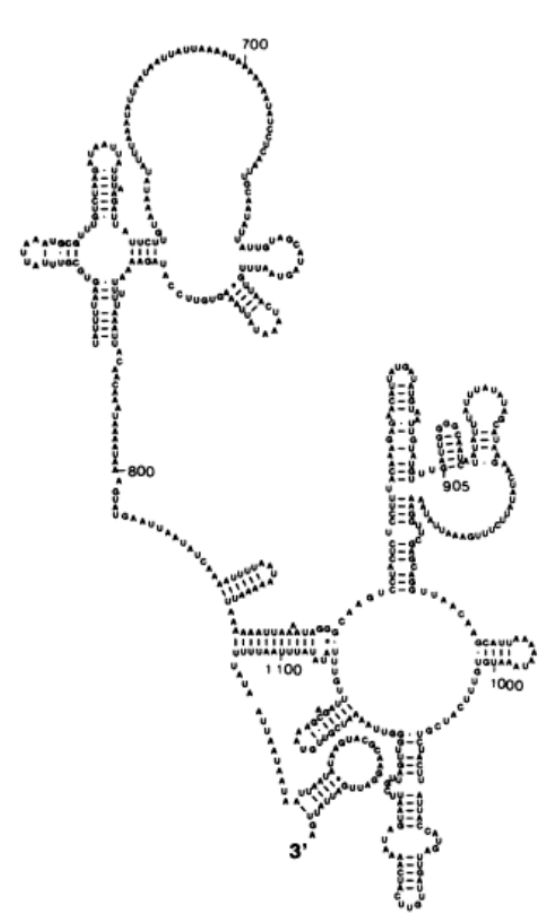
The mt LSU-rRNA in diplonemids ctnd



- No evident similarity to the *Trypanosoma* rRNA structure.



*D. papillatum*¹



*T. brucei*²

(1) Valach M et al (2014) Nucleic Acids Res 51:6443-6460 (2) Gutell & Fox (1088) Nucleic Acids Res 16 (suppl): r1754