



# Mitochondrial genomics and EPN

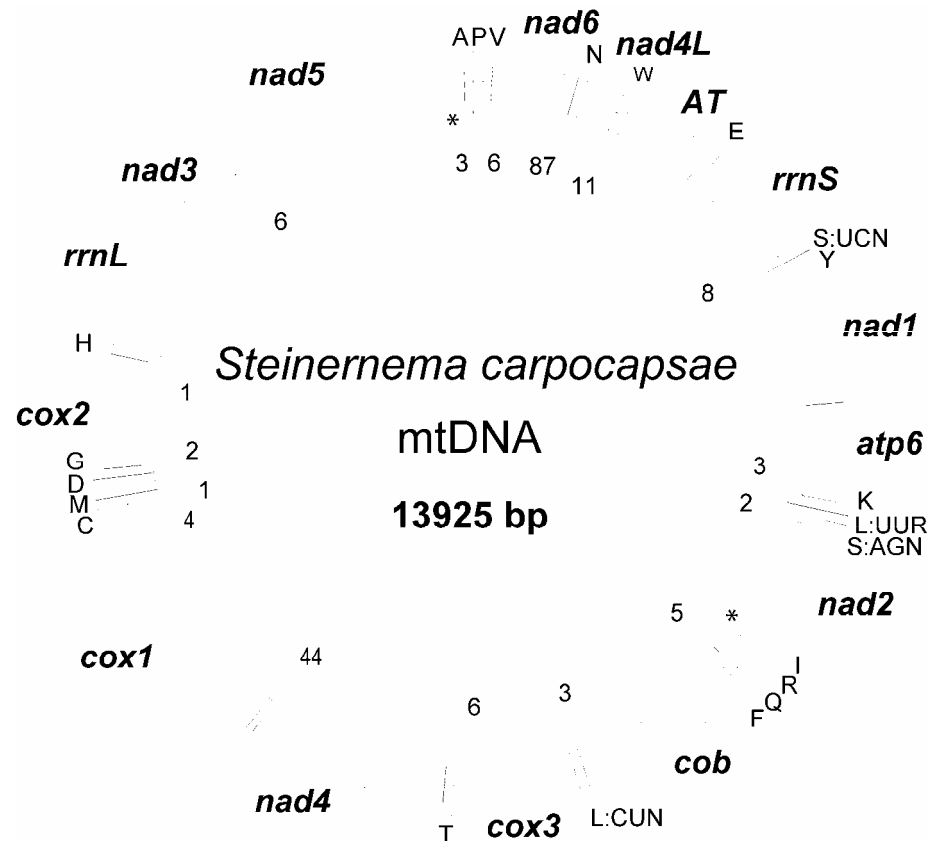
- Mitochondrial genome sequences are widely used in evolutionary studies of many different groups of organisms.
- Mitochondria provide a closed system within which several genetic mechanisms are currently being studied.
- Mitochondrial genomics in EPN may shed light on ecological aspects of mitochondrial DNA evolution, and to assess its role in symbiosis, pathogenesis, and stress response.

# The complete mt genome of *Steinernema carpocapsae*

- Mitochondria were isolated from a 1 ml pellet of females of isofemale descendance
- After mtDNA extraction, conserved primers were used to amplify almost the whole mitochondrial genome of *S. carpocapsae* that was sequenced by a shot-gun strategy
- This procedure minimize the risk of amplify and sequence NUMTs

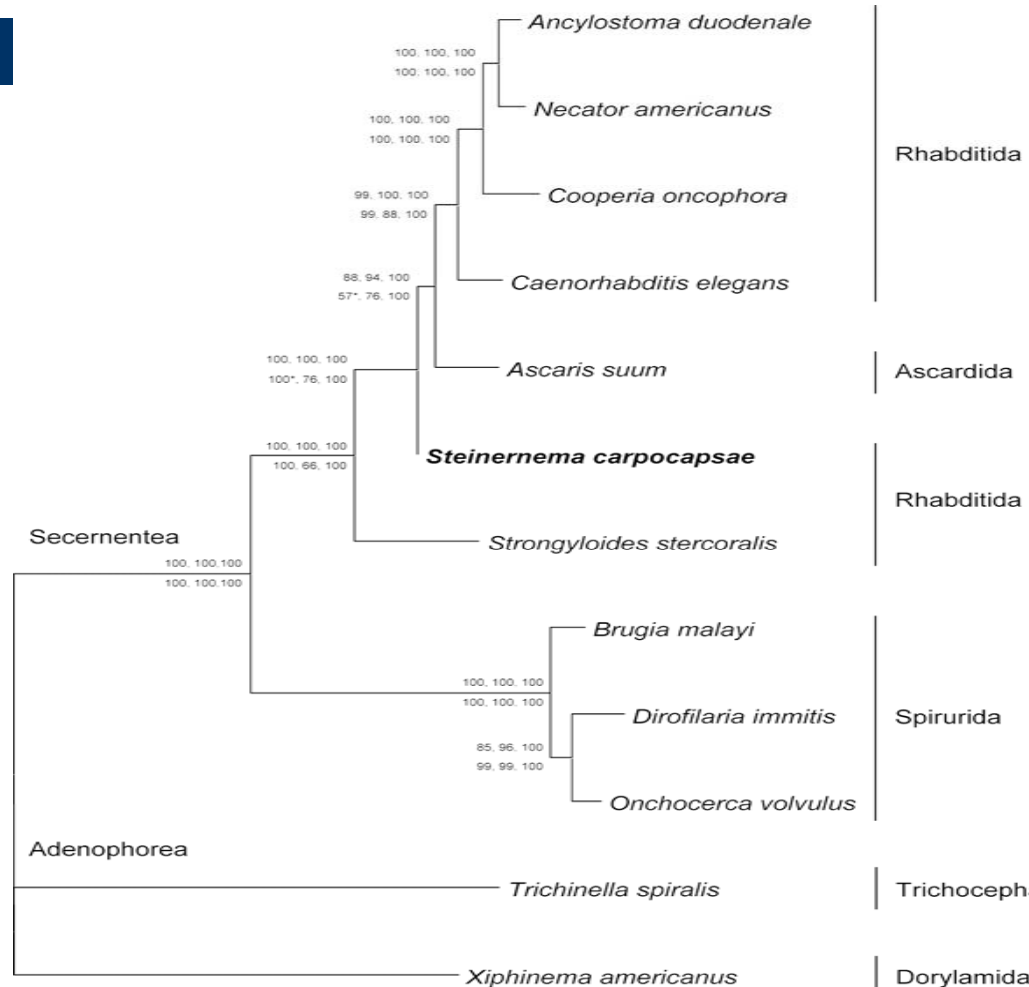
# The complete mt genome of *Steinernema carpocapsae*

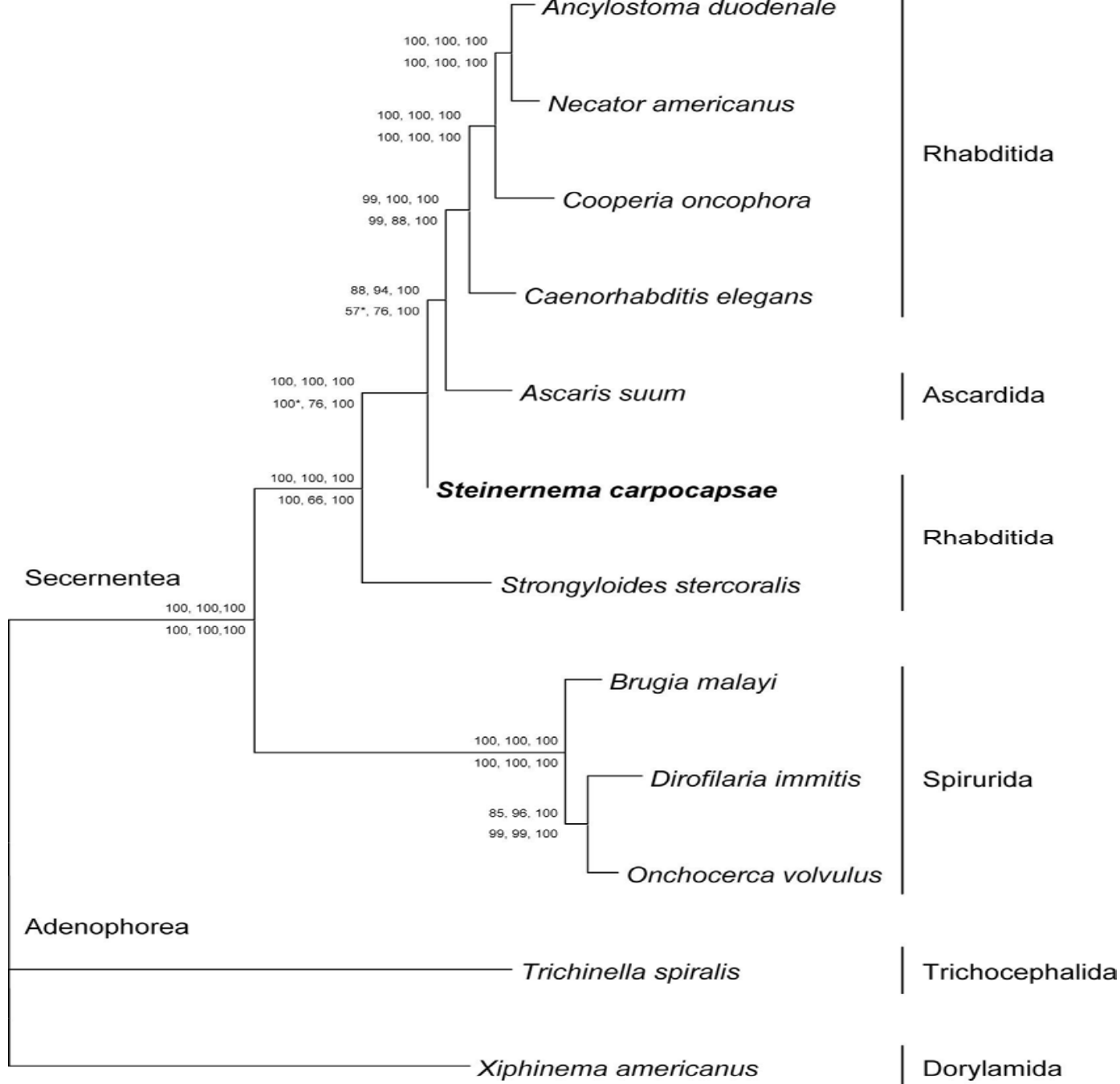
- Comprises 13925 bp
- 12 protein coding genes
- 2 genes for rRNA sub.
- 22 genes for tRNAs
- no gene for ATPase 8
- all genes transcribed in the same direction



# Mitochondrial genomes - phylogenetic reconstruction

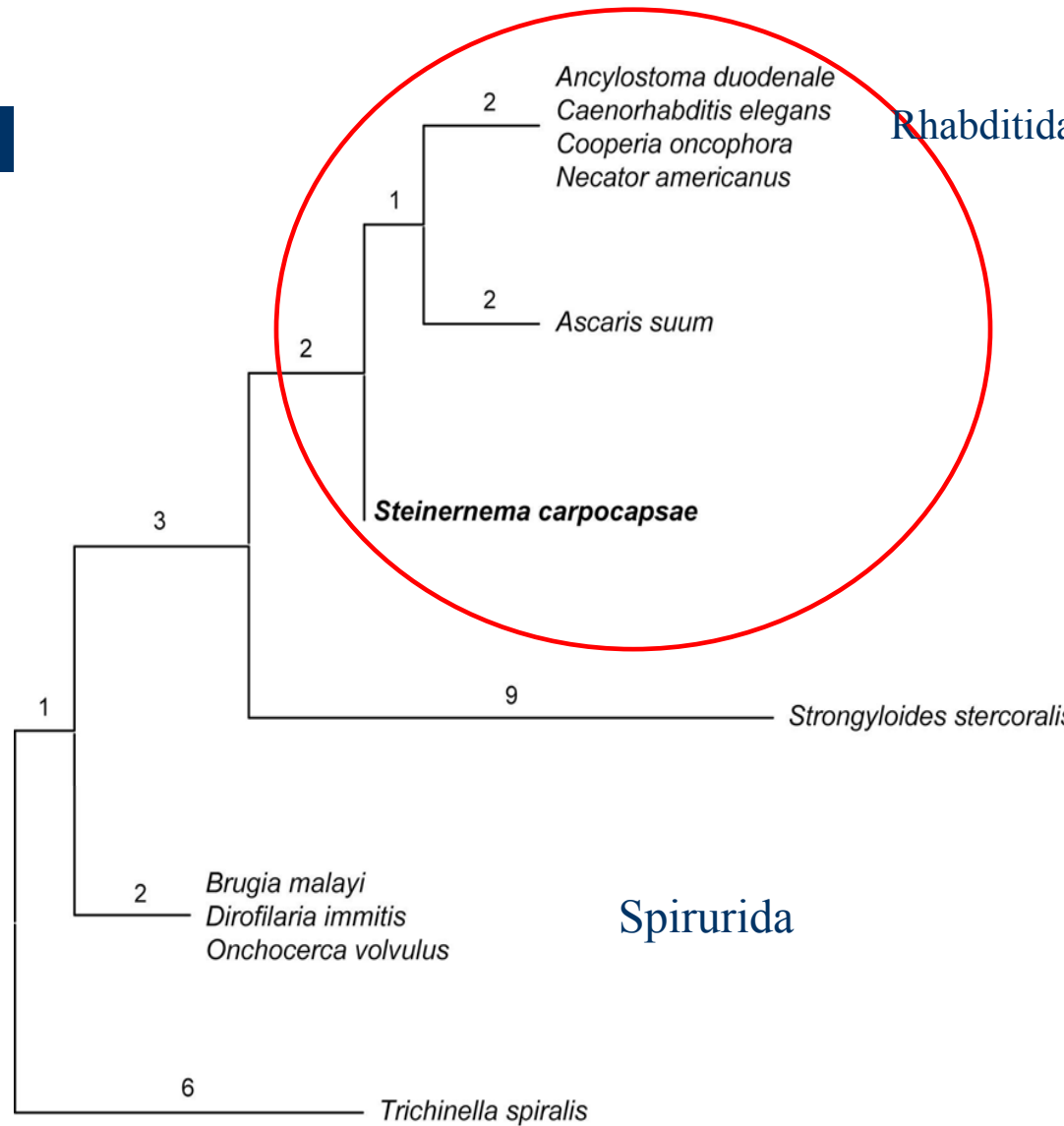
- Bayesian tree
- Protein alignment
- CpREV model (Adachi *et al.* 2000)
- Adenophoreans as outgroups





# Mitochondrial genomes - phylogenetic reconstruction

- Tree constructed from gene arrangement data
  - GRIMM (Tesler, 2002)
  - MGR (Bourque and Pevzner, 2002)

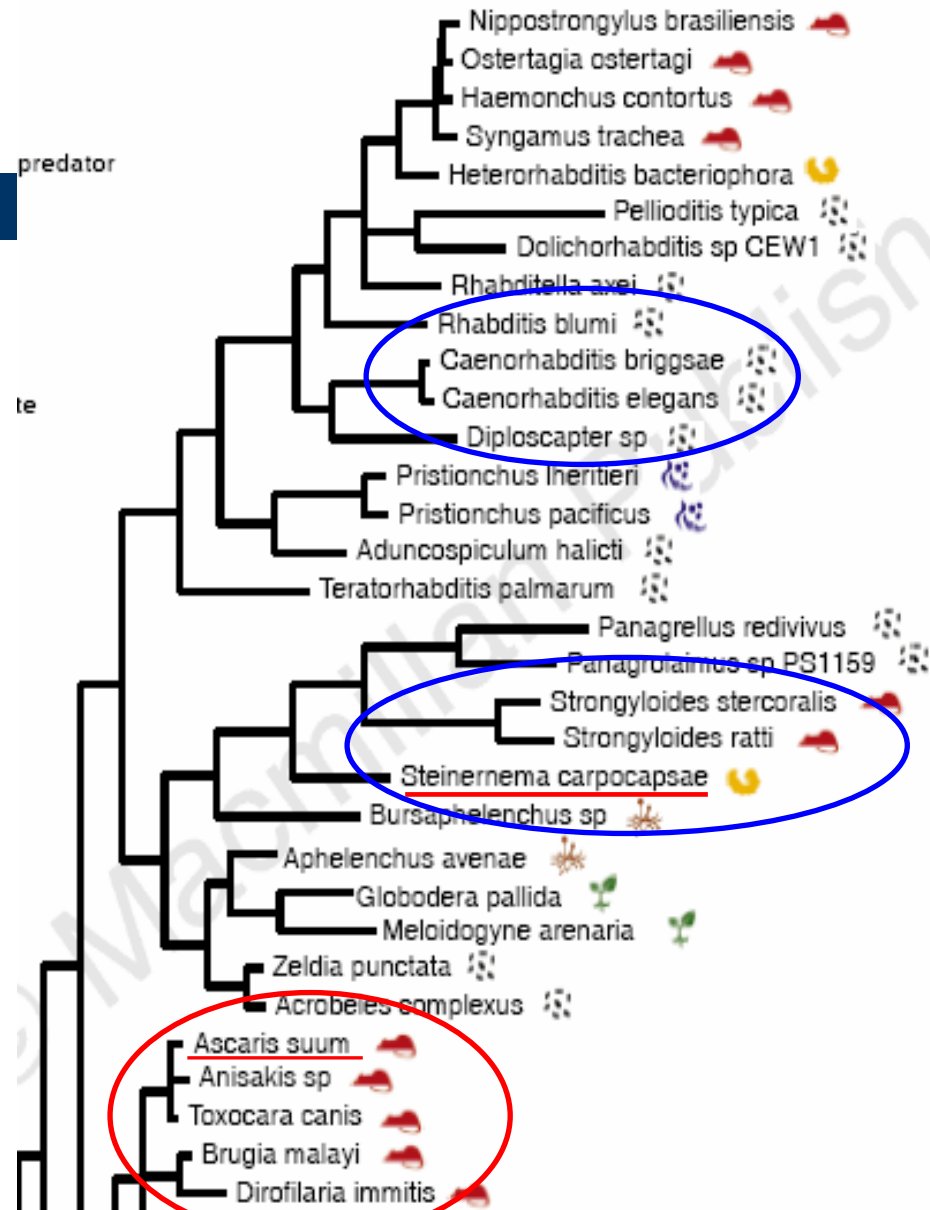


# mtDNA vs 16S

## phylogenies

### 16S phylogeny

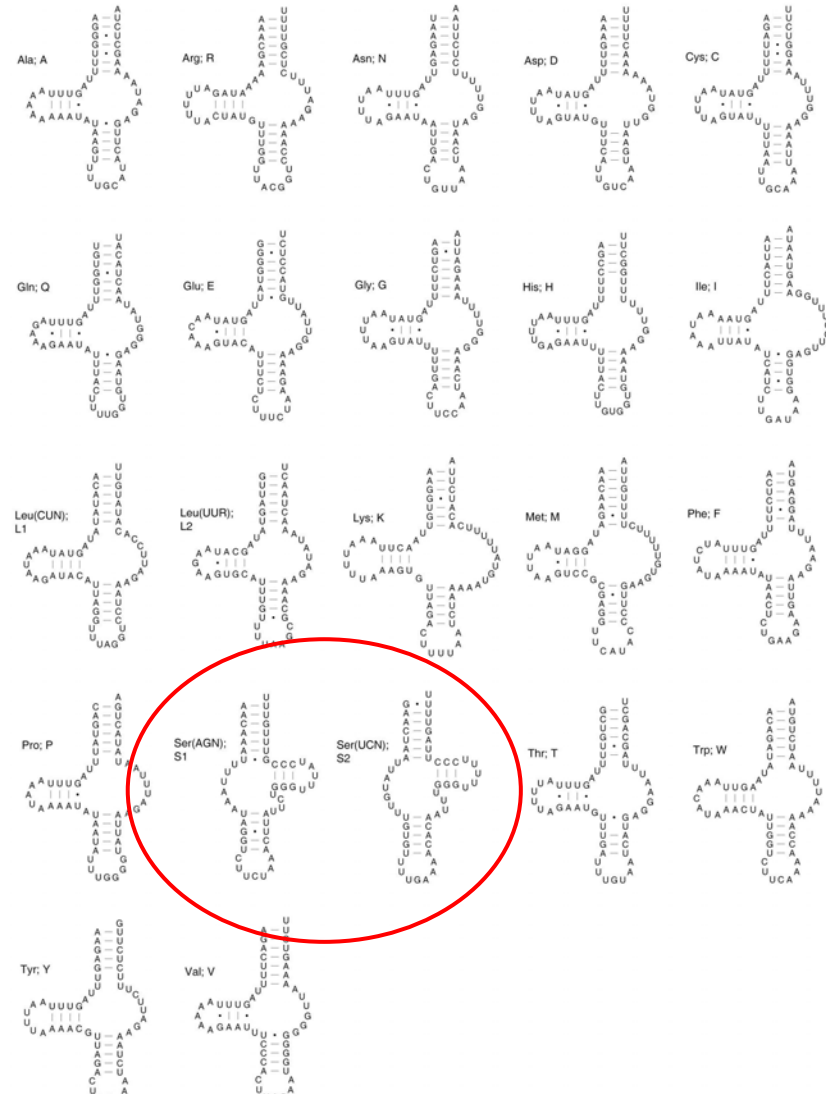
- *S. carpocapsae* more related with *S. stercoralis*
- *A. suum* grouped with Spirurida
- Blaxter *et al.* 1998



# Insights into tRNA editing

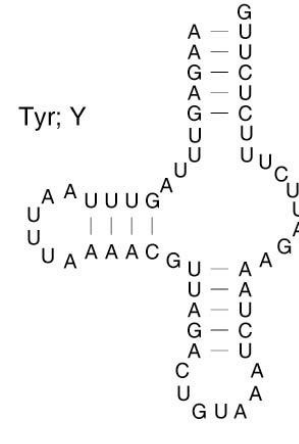
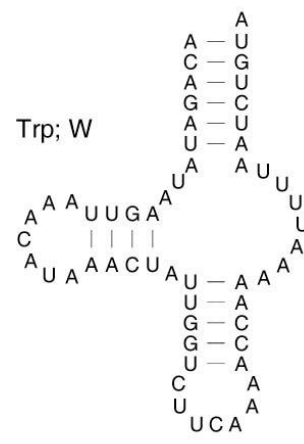
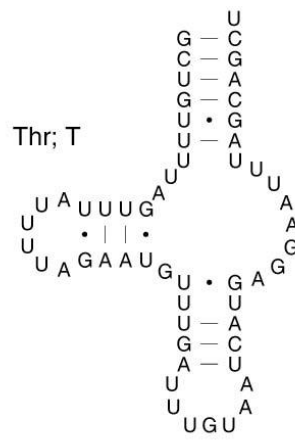
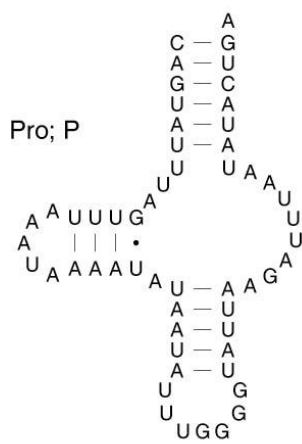
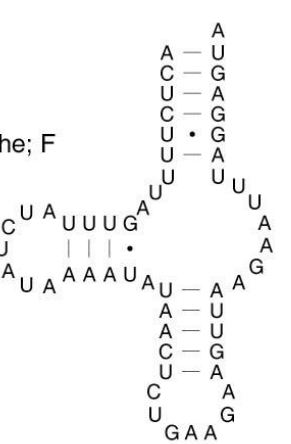
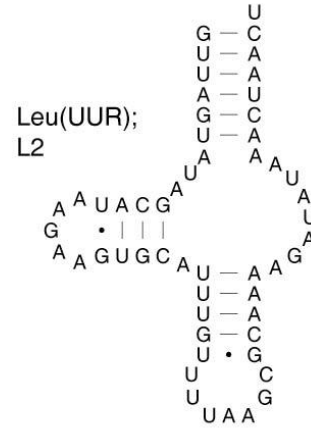
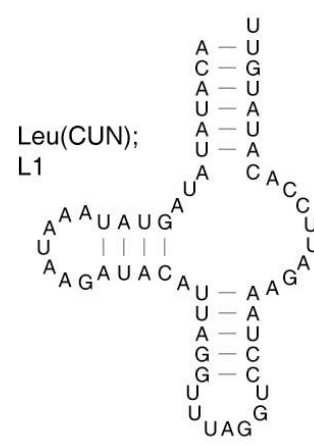
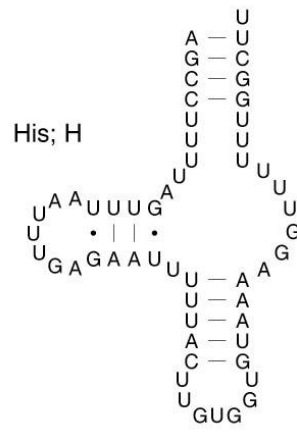
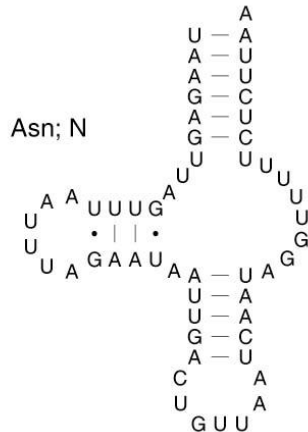
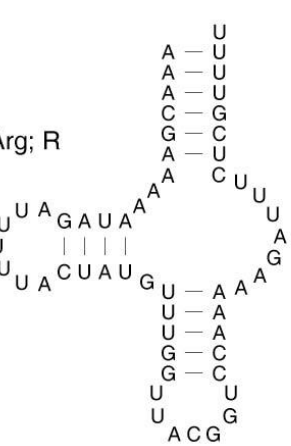
## mt-tRNAs found in *S. carpocapsae*

- 22 tRNAs
- Typical of Secernentea
- 20 lacked T $\psi$ C arms
- 2 (for Serine) lacked DHU arms



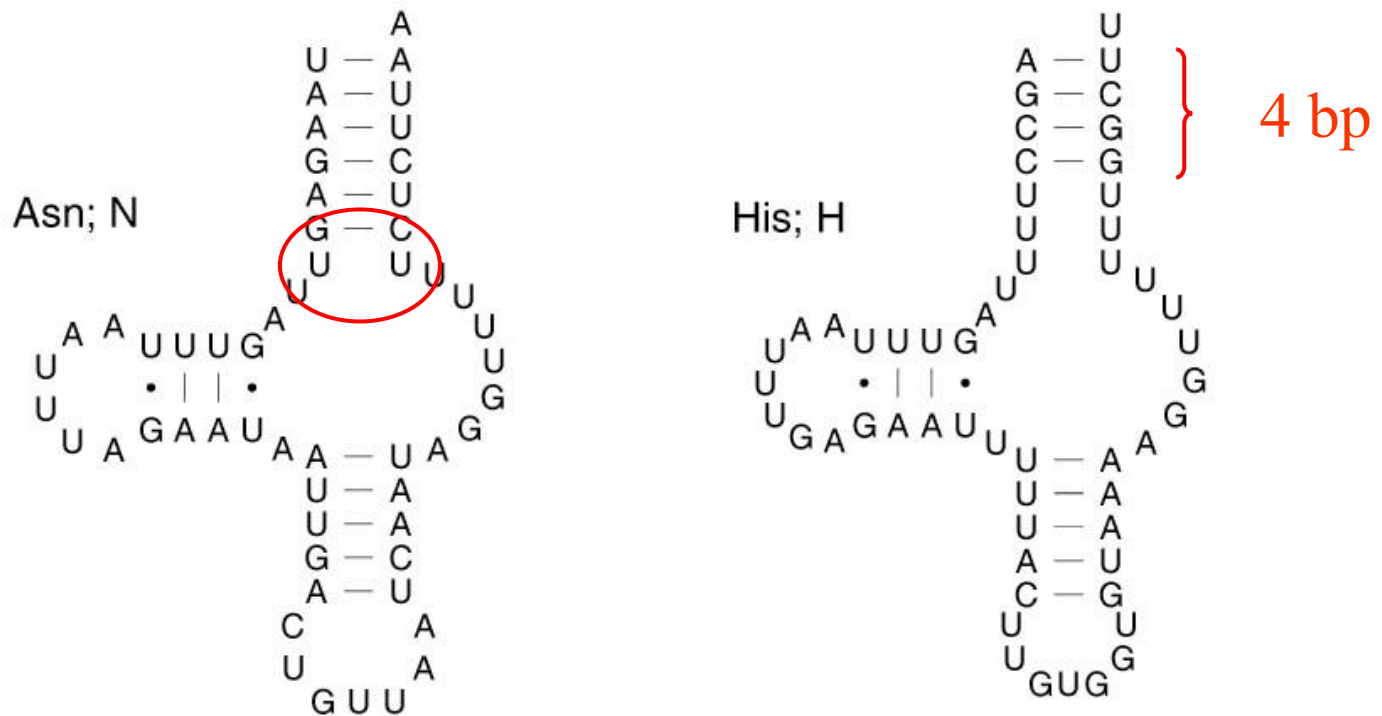
# Mismatches in mt-tRNAs

Thirteen mismatches in the aminoacyl acceptor stem in 10 tRNAs



# Mismatches in mt-tRNAs

The most common mismatch was between nucleotides 7 and 66



tRNA for Histidine presented the shortest acceptor stem

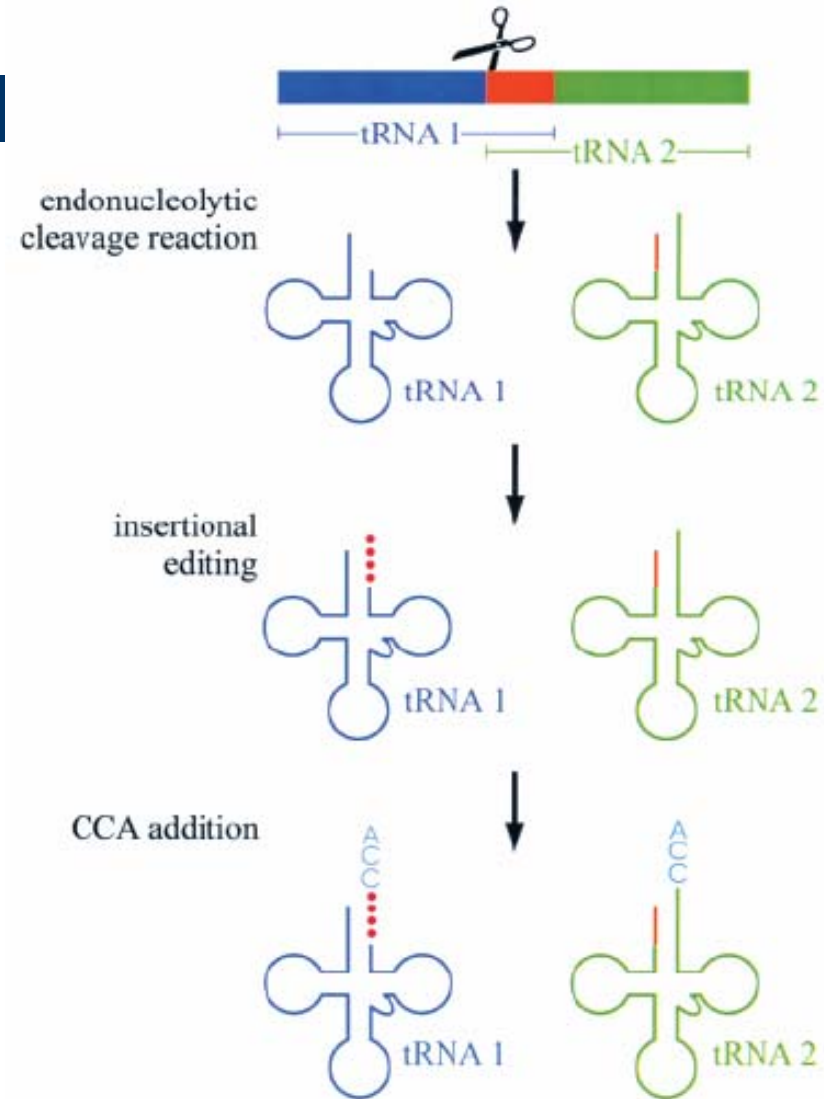
# tRNA editing in nematode mitochondria??

- A well-matched acceptor stem is important for defining RNA structure, directing tRNA processing, and assisting tRNA recognition by aminoacyl-tRNA synthetase (Lavrov et al. 2000).
- Hu *et al.* 2003 proposed that mismatches in nematode mt-tRNAs may be corrected by an editing mechanism.
- However... the mechanisms currently hypothesized do not fit well with observed mismatches in nematode mt-tRNAs

# tRNA editing mechanisms

## Insertional Editing

Processing and 3'-Terminal Editing of an Overlapping tRNA Precursor Molecule

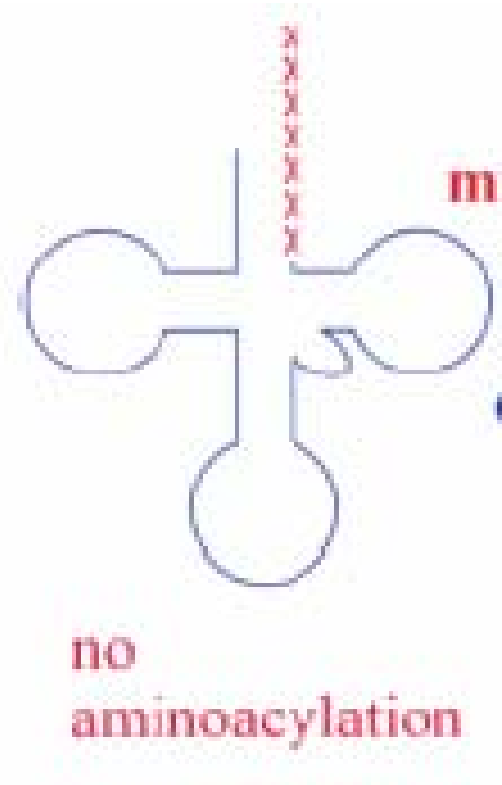


# tRNA editing mechanisms

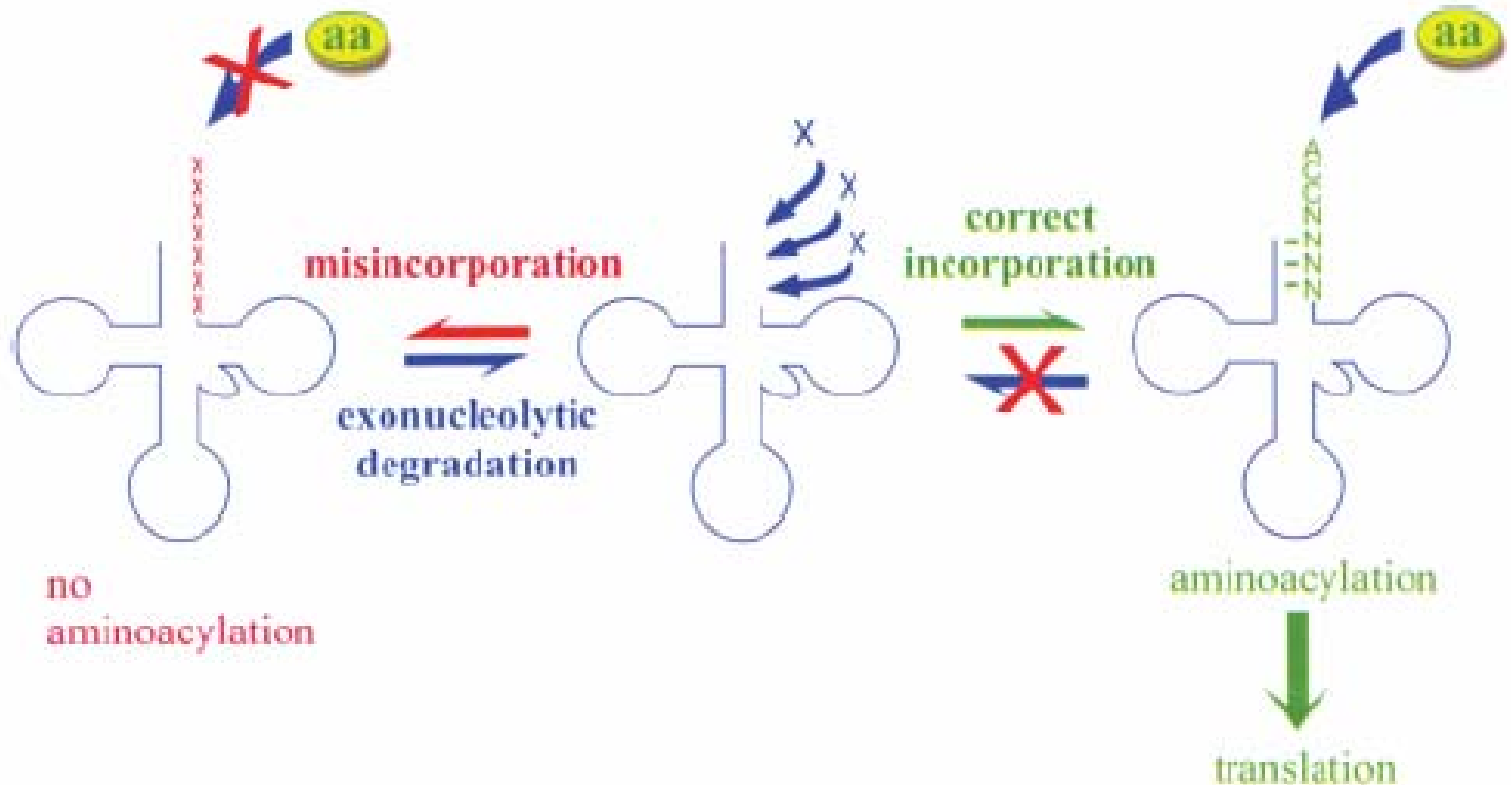
3'-Terminal Editing of tRNA  
Precursor presenting mismatches in  
acceptor stem

Template independent:

- Polyadenylation (Yokobori and Paabo, 1995; 1997)
- Dynamic repair model (Reichert and Mörl, 2000)



# tRNA editing mechanisms



Dynamic repair model (Reichert and Mörl, 2000)

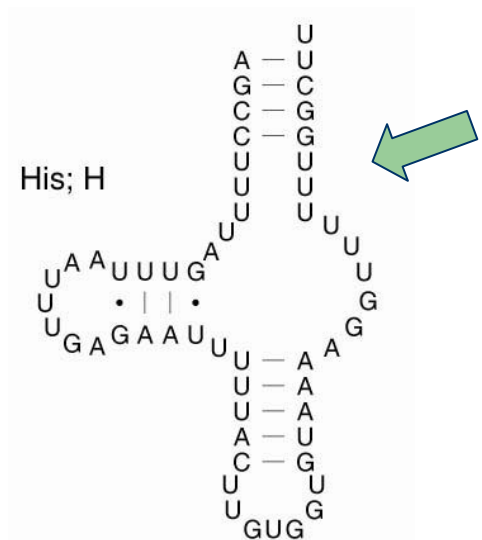
# tRNA editing mechanisms

- Other proposed mechanisms may be template dependent:
  - 3' editing by an RNA-dependent RNA polymerase (Lavrov *et al.* 2000).
- All proposed methods involve repair of mismatches at the end of acceptor stems



# tRNA editing mechanisms

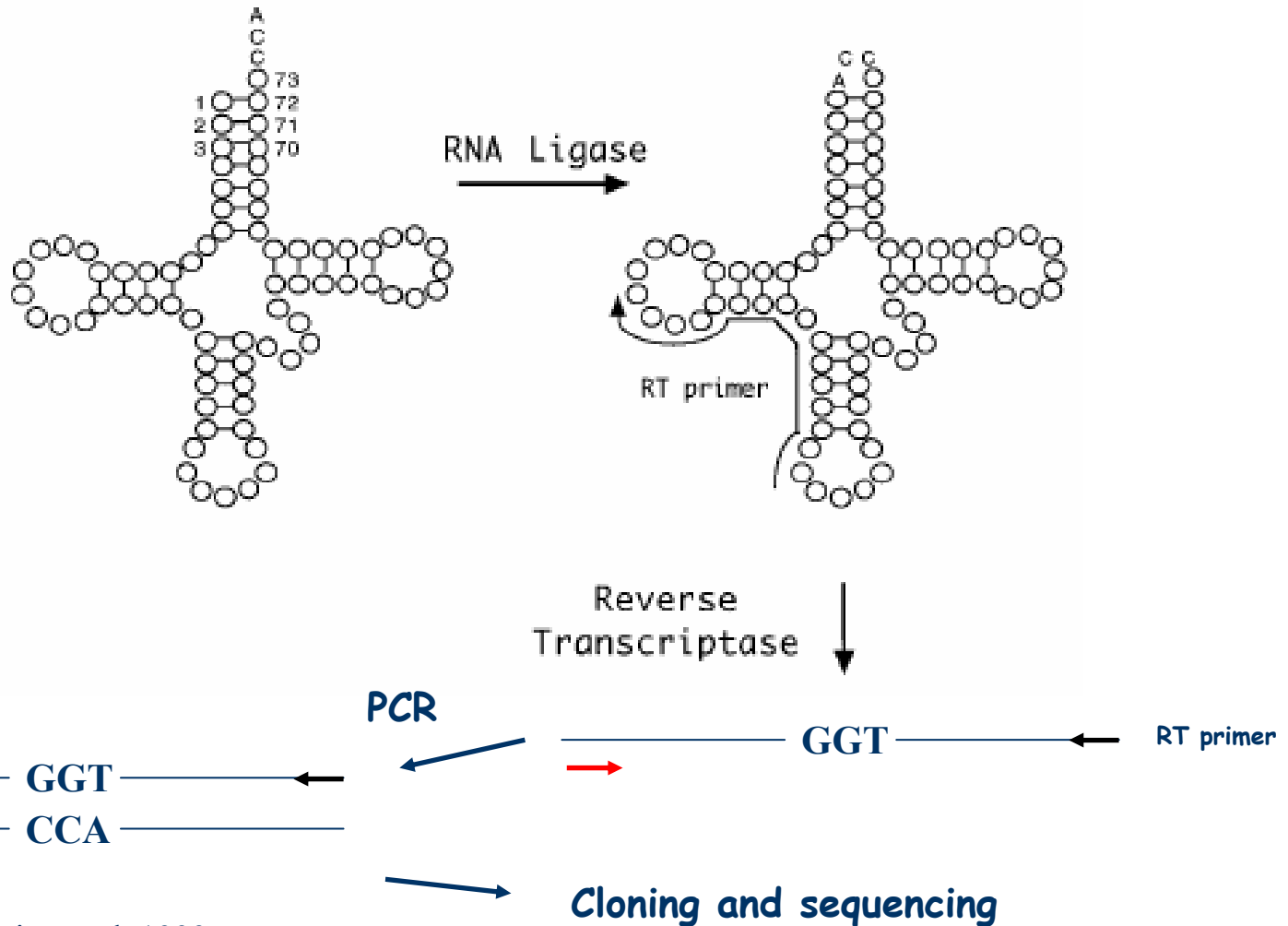
- Other proposed mechanisms may be template dependent:
  - 3' editing by an RNA-dependent RNA polymerase (Lavrov *et al.* 2000).
- All proposed methods involve repair of mismatches at the end of acceptor stems



# mt-tRNA editing in *Steinernema carpocapsae*??

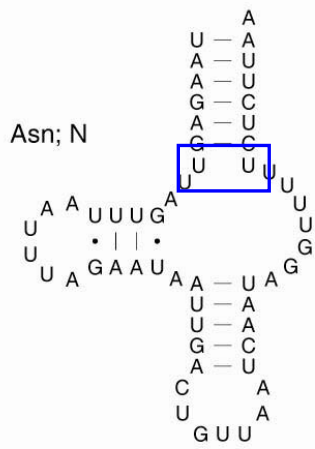
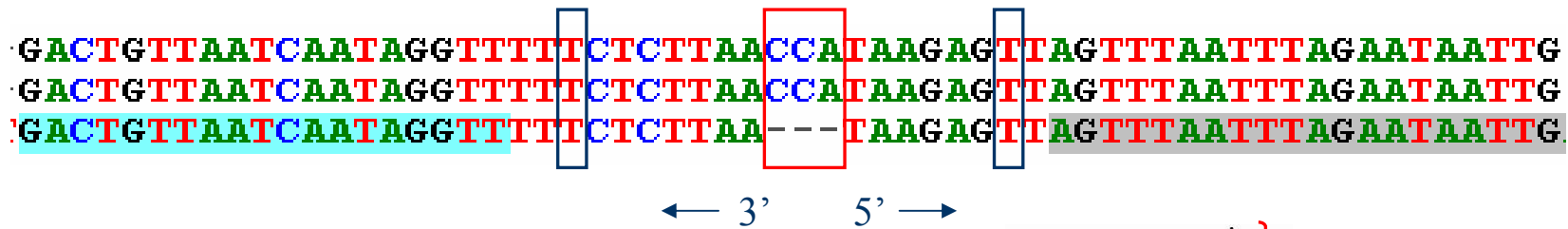
- tRNA extraction either from single nematodes (half) or from a pool of nematodes of isofemale descendance
- Circularisation of tRNAs by T4 RNA ligase
- Retrotranscription of tRNAs with specific primers
- cDNA amplification, cloning and sequencing

# mt-tRNA editing in *Steinernema carpocapsae*??

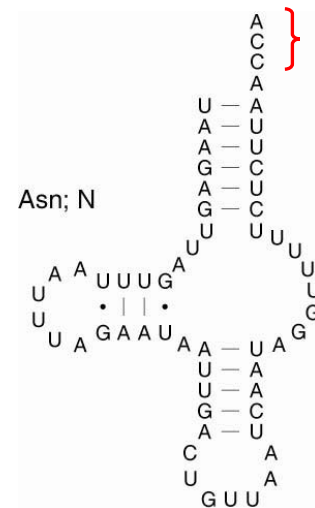


# Preliminary results

- gDNA and cDNA sequences of tRNA<sup>Asn</sup>



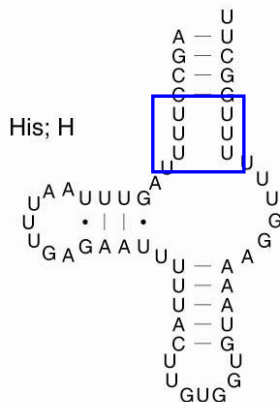
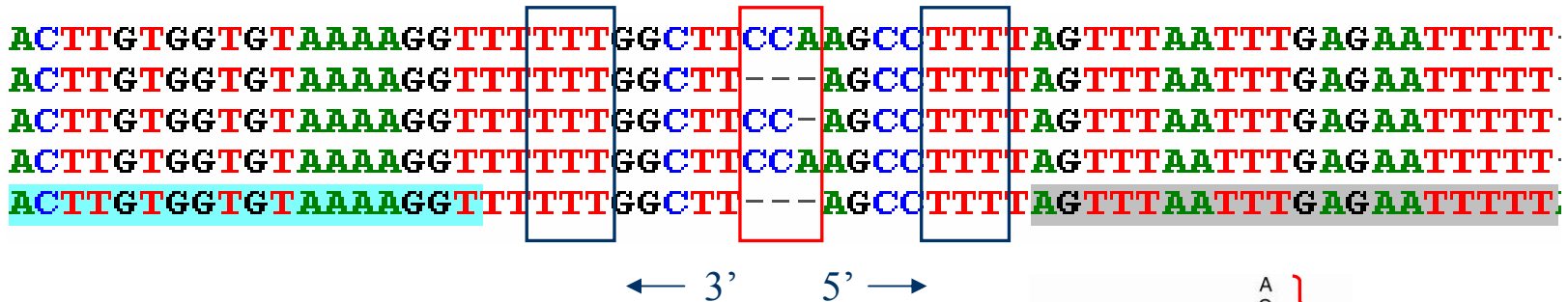
gDNA



cDNA

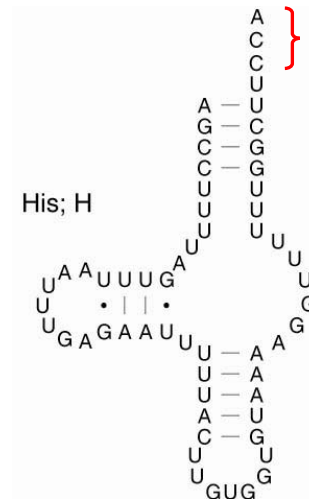
# Preliminary results

- gDNA and cDNA sequences of tRNA<sup>His</sup>



gDNA

=



cDNA

# Discussion

- These preliminary results indicate the absence of tRNA editing in nematode mitochondria.
- Nevertheless, the work of Laforest *et al.* (2004) shows that tRNA editing is not required for addition of the CCA tail by nucleotidyl transferase, and the CCA tail does not appear to be necessary for editing. These two processes, CCA addition and editing, seems to function independently of each other.
- We have sequenced few clones and further work is needed to validate our preliminary results.

# Discussion

- On the other hand, a well-matched acceptor stem is important for processing and function of tRNAs
- If confirmed, our results imply that short acceptor stems can be fully functional for protein synthesis
- Modified enzymes may be involved in tRNA processing and charging



# Conclusion

- Our results shows that tRNA editing mechanisms may be acting only when the end of the acceptor stem needs to be restored.
- Very short aminoacyl acceptors stems may be functional for protein synthesis in nematode mitochondria.

# Acknowledgements

- **FCT** Fundação para a Ciência e a Tecnologia  
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR
  - POCTI/AGR/41664/2001
  - SFRH/BPD/13256/2003