Presence and Absence of COX8 in Reptile Transcriptomes

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Mitochondria are organelles found in most eukaryotic cells that are responsible for supplying chemical energy in the form of adenosine triphosphate (ATP). Located in the mitochondria inner membrane, the electron transport chain (ETC) is series of proteins that shuttle electrons to synthesize ATP. The different components of the ETC are well conserved among eukaryotes in general and among vertebrates in particular. Previous studies have shown that COX8, a relatively small subunit of the ETC was important for optimal mitochondrial performance. Interestingly, several lizard species have apparently lost the gene encoding for this protein. The goal of this research was to assess the phylogenetic extent of the loss of COX8. To do so we looked for traces of the *COX8* coding gene using bioinformatic protocols. These databases included publicly available genomes and transcriptomes, as well as several reptilian transcriptomes from our lab. Our results indicate that COX8 has apparently been lost multiple times during the evolution of amniotes, at least once among turtles, once among snakes, and once among lizards. All these losses have been recorded among ectotherm animals, suggesting the lower metabolic demands of these animals allow them to perform well in the absence of COX8.

Introduction

Mitochondria are organelles found in most eukaryotic cells that are responsible for supplying energy in the form of adenosine triphosphate (ATP) during aerobic respiration. The electron transport chain (ETC) is series of proteins that shuttle electrons via redox reactions, generating a proton gradient across the internal membrane of the mitochondria, with oxygen as the final electron acceptor and ATP as ultimate product (Figure 1). The major proteins in the system are NADH dehydrogenase, succinate dehydrogenase, cytochrome b, and cytochrome oxidase c, all of which assemble into multisubunit complexes. Unsurprisingly, mitochondria appear to be involved in several process related to aerobic performance such as adaptation to life in high altitudes and athletic performance. In addition, from a medical standpoint, mitochondria have been implicated in several human diseases, including mitochondrial disorders and cardiac dysfunction, and may play a role in the aging process.

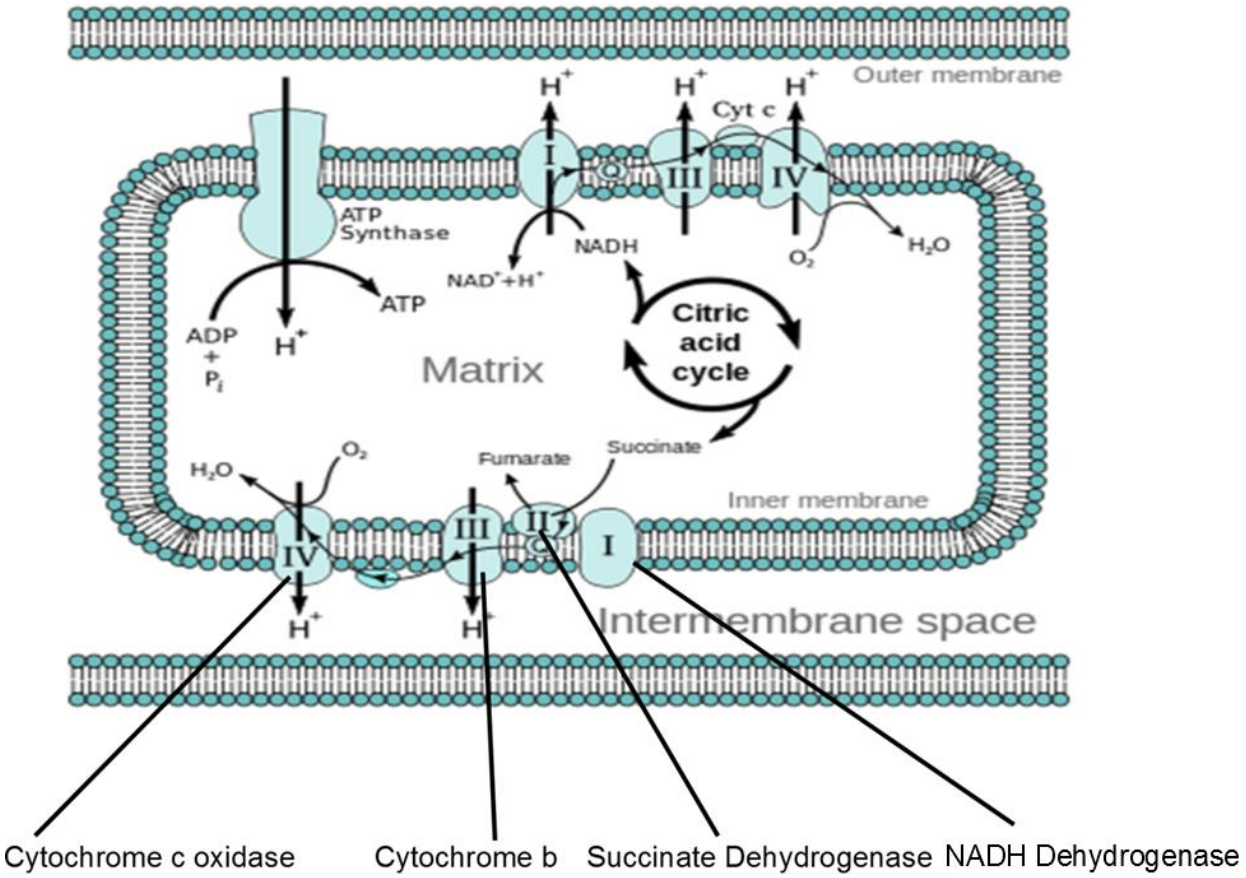

Figure 1. The electron transport chain, a series of protein complexes that transport electrons across a membrane to ultimately produce ATP. COX8 is found within complex IV, also known as cytochrome c oxidase.
(http://upload.wikimedia.org/wikipedia/commons/8/89/Mitochondrial_electron_transport_chain)

Among vertebrates, different studies in mammals and birds suggest that there are clear differences in selective pressure between animals with different lifestyles (Heffner, R. S. 2004) (Ruxton, G. D. et al. 2004). Interestingly, one particular subunit, cytochrome oxidase c subunit 8 (COX8) appears to be missing from several reptiles: chameleons, iguanids, and anoles (Bar-Yaacov, et al. 2013). Physiological and biochemical studies suggest that COX8 is important for peak metabolic performance (Patterson, T. E. et al. 1986). Thus, it was interest to explore the evolutionary extent of the loss of COX8 in more detail. Specifically, we searched for COX8 in homologous genomes and blood transcriptomes of several amniotes to establish when COX8 was lost and how the presence and absence of COX8 relates to differences in natural history among the lineages studied. Our bioinformatic searches failed to identify COX8 homologues in multiple lineages that are not closely related, suggesting that it was probably lost multiple times. However, this question has not been explored in a systematic approach, and a systematic assessment of the distribution of COX8 among amniotes is missing.

Objective

The goal of this research was to explore the evolutionary distribution of COX8 among amniotes in a systematic manner, to map the losses onto the organismal tree and explore connections between the presence of COX8 and the metabolic regime of the different species studied.

Methods and Materials

Sample collection and RNA-Seq

We extracted blood from twelve Squamate reptiles (Texas blind snake, red eyed crocodile skink, tokay gecko, prairie rattlesnake, yellow racer, savannah monitor, blonde hognose snake, bearded dragon, boa constrictor, chameleon, Asian vine snake, and gold tegu). From the blood we extracted the RNA using Trizol®, based on the manufactures protocol and mRNA was isolated using oligo dT beads. The mRNA libraries were prepped using a Nextera directional RNA-Seq library prep kit (Caruccio, N. 2011) for sequencing on two 2x100 Illumina HiSeq lanes (Caporaso, J. G et al. 2012). Transcriptomes of each species were assembled using Trinity (Grabherr, M. G. et al. 2011).

Computation analyses

We obtained the COX8 isoforms A and B protein sequence of human and cow, respectively from Ensembl (UniProt Consortium. 2008). We also gathered the mitochondrially encoded cytb gene from other genomes of each of the reptiles we queried and the nuclear encoded cytc gene from the green anole to use in control searches.

First, the snake transcriptomes were queried for COX8 isoforms using BLASTx(Altschul, S.F. et al. 1997) (Basic Local Alignment Search Tool that translates nucleotide sequences into amino acid sequences and compares them to a protein database) and when contigs coding for COX8 were found within the snakes they were extracted and added to the BLAST database in order to search for the most similar COX8 sequences in the lizards. The remaining reptile transcriptomes were queried against new database, and were extracted when found.

Afterwards, the control protein subunits were compared to each of the transcriptomes using BLASTx. For each transcriptome, the resulting best hits were extracted, and we recorded presence absence among species. Chameleon did not have any hits when searched with the minimum E-value set at 1e-10, so it was searched again without a minimum E-value and the best match for COX8 was extracted.

In order to validate that our findings were accurate, the extracted contig sequence best hits of COX8 from *Chameleon, Leptotyphlops,* and *Tupinambis* were used in a reciprocal BLAST against the NCBI database (Pruitt, K. D. et al. 2007)and aligned with the best match. *Tupinambis* and *Lytotyphlops* COX8 were compared with Blastx against the protein database, *Chameleon* was compared using BLASTn (Altschul, S.F. et al. 1997) (Basic Local Alignment Search Tool that compares a nucleotide sequence to a database of nucleotide sequences).

Results

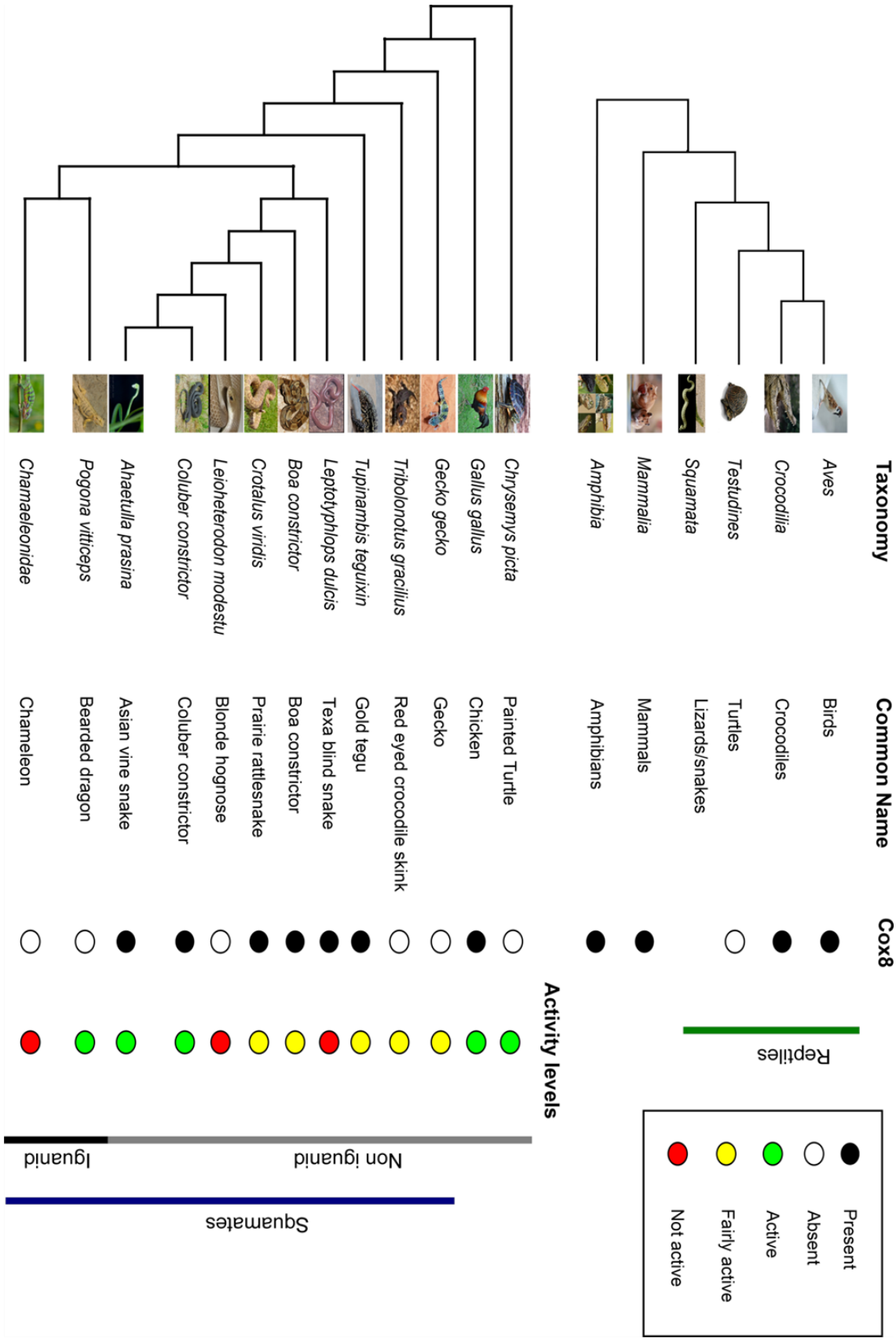
Taxonomy
Common Name
Cox8
Activity levels
Aves
Crocodilia
Testudines
Squamata
Mammalia
Amphibia
Chrysemys picta
Gallus gallus
Gecko gecko
Tribolonotus gracilius
Tupinambis teguixin
Leptotyphlops dulcis
Boa constrictor
Crotalus viridis
Leioheterodon modestu
Coluber constrictor
Ahaetulla prasina
Pogona vitticeps
Chamaeleonidae
Birds
Crocodiles
Turtles
Lizards/snakes
Mammals
Amphibians
Painted Turtle
Chicken
Gecko
Red eyed crocodile skink
Gold tegu
Texa blind snake
Boa constrictor
Prairie rattlesnake
Blonde hognose
Coluber constrictor
Asian vine snake
Bearded dragon
Chameleon
Reptiles
Present
Absent
Active
Fairly active
Not active
Squamates
Non iguanid
Iguanid

Figure. 2 Phylogenetic tree of eukaryotes and reptiles, respectively, illustrating Order Squamata in relation to other eukaryotes, and further showing iguanids in comparison to other squamates. Activity levels are based on physical movement as well as any internal processes that affect metabolism, such as making venom, being endothermic, etc.

Name	Common Name	COX8 present
Homo sapien	Human	Yes
Xenopus laevis	African clawed frog	Yes
Gallus gallus	Chicken	Yes
Alligator sinensis	Chinese alligator	Yes
Chrysemys picta	Painted turtle	No

Table 1. Depicting COX8 presence in eukaryotes

Name	Common Name	Cox8 Results	Cytb (control)	Cytc (control)
Leptotyphlops dulcis	Texas blind snake	Yes	Yes	Yes
Tribolonotus gracilis	Red eyed crocodile skink	No	Yes	Yes
Gecko gecko	Tokay gecko	No	Yes	No
Crotalus viridis	Prairie rattlesnake	Yes	Yes	Yes
Coluber constrictor	Yellow racer	Yes	Yes	Yes
Varanus exanthematicus	Savannah monitor	Yes	Yes	Yes
Leioheterodon modestus	Blonde hognose snake	No	Yes	Yes
Pagona vitticeps	Bearded dragon	No	Yes	Yes
Boa constrictor	Boa contrictor	Yes	Yes	Yes
Chameleo chameleon	Common chameleon	No	Yes	Yes
Aetualla prasina	Asian vine snake	Yes	Yes	Yes
Tupinambis teguixin	Gold tegu	Yes	Yes	Yes

Table 2. Depicting our findings of COX8, cytb, and cytc in our transcriptomes.

After querying the transcriptomes, we could not find the COX8 coding contigs in both of our iguanids (*Chameleo, Pogona*), and three other reptiles (*Tribolonotus, Gecko, Leioheterodon*) (Figure 2, Table 2) while using the NCBI database we could find COX8 for other orders (Table 1).

The COX8 coding contigs that were extracted were not highly conserved between the reptiles and mammals. For instance the *Tupinambis* percent identical matches (percent identity) when compared to the human COX8 was 43.9%, with an E-value (Expect value, the number of nucleotide or amino acid matches expected to occur by chance) of 2e^-12, and a length of 41 amino acids. While the length seems short, the COX8 gene is fairly short, only about 250 nucleotides long. When compared to human COX8 protein isoforms, *Leptotyphlops* COX8 percent identity was 43.9, E-value was 2e-11, and length was 41 . When compared to human COX8 protein isoforms, *Leptotyphlops* COX8 percent identity was 43.9, E-value was 2e-11, and length was 41 amino acids. However, when compared against the NCBI database, the *Tupinambis* and *Leptotyphlops* were both matched to *Pabio anubis* COX8 (olive baboon) with a percent 56%, with the E-value of 7e^-07, and a length of 41 amino acids.

The chameleon COX8 contig matched up with our human and mouse contigs with percent identity 34%, length 50, E-value 6e-6 and when compared to the NCBI database did not match up to anything in the electron transport chain, and it instead matched up to a protein associated with brain tumors with a percent identity 83%, length 146 nucleotides, and E-value 5e-116.

The control tests determined that cytochrome b was present in all transcriptomes with a percent identity of 75% and higher, E-values of 4e-7 and smaller, and lengths of 484 nucleotides and higher. Cytochrome c was conserved with percent identity 81.37 and higher, E-value 1e-29 and smaller, and lengths of 100 nucleotides and higher. Cytochrome c was not found in the *Gecko*, which is most likely due to low coverage of our data

Conclusion

Like in previous studies,we could not detect COX8 homologues of the iguanid transcriptomes assayed nor in other groups (Tribolonotus, Gecko, Leioheterodon). In recent phylogenetic reconstructions, Iguania appeared to be most closely related to the Anguimorpha, the group that includes monitor lizards, Gila monsters and alligator lizards. We identified COX8 in the savannah monitor and most of the snakes, thus can confidently say the true loss of COX8 only occurred in the Iguania lizards and not in the common ancestor of Iguania and Anguimorpha. The apparent loss of COX8 in other groups could be explained by a secondary independent loss of the gene as turtles are also missing the COX8 gene. However, all the transcripts expressed in the blood may not be represented here and it is possible that the gene is present in all Squamates except Iguania but was not detected. Alternatively, the COX8 gene was not assembled correctly or only partially represented and was not discovered by BLAST’s search algorithm. So far COX8 has only been lost in ectotherms.

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