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Molecular Search and Characterization of the Amphibian Uncoupling Protein 1

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RESUMEN

Durante la regulación térmica corporal y oxidativa, las proteínas desacopladoras rompen el flujo de electrones a través de la membrana mitocondrial interna. Dentro de esta familia de secuencias, se encuentra la termogenina (UCP1 por sus siglas en ingles), una de sus copias homologas. Esta, logra disipar la energía que al no ser convertida en ATP se transforma en calor realizando termogénesis molecular. Además, este proceso regula el equilibrio de oxido reducción intracelular siendo un proceso clave durante el estrés oxidativo. Siendo así, que la actividad de esta proteína esta mediada por aminoácidos clave en su estructura terciaria, los cuales permiten el movimiento de iones hidrogeno a través de su poro. De la misma manera, su estructura posee otros residuos que permiten su control inhibitorio, proceso que se realiza por medio de la unión de nucleótidos que bloquean la entrada de este canal. Es de esta manera, que la actividad desacoplante, variará dependiendo de los residuos característicos de cada copia, siendo la termogenina la de mayor actividad termogénica. Por consiguiente, en el presente estudio se procedió a analizar los distintos residuos que conforman la estructura funcional del homólogo anfibio de la UCP1, evidenciando la conservación topológica de esta copia. Fue mediante la predicción de secuencias y estructuras que aminoácidos esenciales para la unión de nucleótidos y transporte de protones fueron hallados en las mismas posiciones que su copia funcional en mamíferos. De la misma forma, la comparación en cuanto a los modelos estructurales entre estos grupos reveló una estrecha semejanza respecto a su topología terciaría. Siendo así, nuestros resultados demuestran la cercana relación entre la UCP1 funcional de mamíferos y su homólogo en anfibios; permitiéndonos de esta manera predecir una función conservada guiados por su secuencia y estructura. Así pues, nuestro estudio se convierte en un punto de inicio para futuros análisis de mayor profundidad que nos permitirán entender mucho más sobre los mecanismos de control sobre la función de esta proteína en anfibios.

ABSTRACT

During body thermal and oxidative regulation, uncoupling proteins break the proton flux through the inner mitochondrial membrane. Within this family of proteins, the Thermogenin (UCP1 for its acronym in English) is one of the homologous copies. This protein, UCP1, decouples the electron transfer chain and dissipate the energy being turned into ATP is transformed in heat producing molecular thermogenesis. This process regulates the balance of intracellular redox (reduction and oxidation) reactions as a consequence during oxidative stress. The activity of this protein is mediated by key amino acids in its tertiary structure, which allow the movement of hydrogen ions through its pore. Its structure has other residues that allow its inhibitory control, a process that is carried out through the union of nucleotides that block the entrance of this channel. The decoupling activity will vary depending on the characteristic residues of each copy, being the Thermogenin the one with the highest thermogenic activity. In the present study, we analyzed the different residues that constitute the functional structure of the amphibian homologue of UCP1, evidencing the topological conservation of this copy. We predict the coding sequences and the tridimensional structures that essential amino acids have for nucleotide binding and proton transport, they were found and located to be in the same positions as their functional copy in mammals. The comparison of the structural models among these groups revealed a close resemblance at their tertiary topology level. Our results demonstrate the close relationship between the functional UCP1 of mammals and their homologue in amphibians; allowing us in this way, to predict a preserved function guided by its sequence and structure. Our study thus becomes a starting point for future analyzes of greater depth that will allow us to understand much more about the mechanisms of control over the function of this protein in amphibians.

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MOLECULAR SEARCH AND CHARACTERIZATION OF THE AMPHIBIAN UNCOUPLING PROTEIN 1

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Background

Evolutionary adaptations have been the force that push life on Earth to spread in different environments promoting the survival and diversification of species. Different types of adaptations have existed (Ellery, 2004) were DNA/Amino acids mutations are the source to overcome different types of stress. One of the most challenging environmental constrain is low temperature. Various forms of protection against cold environments have risen as evolutionary adaptations, several times in many groups including amphibians (Margesin & Schinner, 1999). Even if amphibians, has been mostly catalogued as ectotherm beings, their presence in cold environments is puzzling as they don't show physical barriers.

Species located principally in temperate regions (Brattstrom, 2015; Witters & Sievert, 2001), have activity peaks during summer and estivation behaviors during non-favorable conditions like winter. In contrast, species that are in neotropical regions and regions where cold is almost permanent like mountain ranges have different periods of activity. For instance, we find amphibians groups like the Strabomantidae and

Craugastoridae that are mainly active during the night, a period where a non-direct heat sources are available (Catenazzi, Lehr, & Vredenburg, 2014; von May et al., 2017). This empirical evidence has suggest the existence of molecular thermogenesis mechanisms in frogs that live and act in permanent cold environments, involving molecules like Thermogenin and Sarcopilin which is mainly active during muscle shivering thermogenesis (Divakaruni & Brand, 2011; Nowack, Giroud, Arnold, & Ruf, 2017).

The Thermogenin is part of the uncoupling proteins (UCPs), members of the mitochondrial carrier family characterized by its protonophoric activity. These proteins are located in the inner mitochondrial membrane (IMM) aside from the respiratory chain complexes. The structure of these mitochondrial membrane carriers is conserved for all the family members, however its amino acids (AA) can vary. This variation will produce different levels of uncoupling and adaptations that will be reflected in the presence of these proteins in tissues in response to physiological states.

UCPs have three domain repetitions composed by six transmembrane helixes connected by five extra-membrane loops (Michael J. Gaudry, Campbell, & Jastroch, 2018; Klingenspor et al., 2008; Kuan & Saier, 1993). The UCPs present different functions that will vary in their activity based on their difference of amino acid sequences and key residues in their structure. Among their functions as chloride and ion transport, the uncoupling activity represents the most important. Here, the cease of the proton gradient across the IMM causes the accumulation of catabolic compounds, which by not being transformed into ATP, release their energy in the form of heat to the cellular environment (Divakaruni & Brand, 2011; Kuan & Saier, 1993).

The activity for the members of this group will vary not only by their sequences, but also depending on the tissue which is expressed. In this way, upon vertebrates, five

different paralogues have been described (UCP1 to 5) (Klingenspor et al., 2008; Ledesma, de Lacoba, & Rial, 2002). All of the paralogues are present in mammals, having UCP1, -4 found in the rest of vertebrates with the exception of birds that have lost their UCP1 during evolution (Saito, Saito, & Shingai, 2008). The UCP5 or Brain Uncoupling Protein (BUP) has been described as a mediator of energy metabolism and oxidative stress in certain areas of the mammalian brain with changes during development. This protein has a fundamental role in neurodegenerative diseases such as Alzheimer and Parkinson and has been extensively studied (M J Gaudry & Jastroch, 2019; Kim-Han & Dugan, 2005). UCP2 and UCP3 have been proposed as the main regulators of energy and oxidative stress in muscle and liver tissues (Frédéric Bouillaud, Alves-Guerra, & Ricquier, 2016). In contrast, the role of UCP4 is poorly understood and has been considered as the ancestral form of all UCP protein sequence members (Borecký, Maia, & Arruda, 2001; Hanák & Jezek, 2001). Among all the described members, UCP1 figures as the one with a fundamental role in molecular heat product, and non-shivering thermogenesis. This protein is expressed in adipose tissue, mainly in brow fat and secondarily in beige. However, is important to point out that this expression could vary among development and species. The present study will analyze in deep the molecular structure of Thermogenin or Uncoupling protein 1 (UCP1) in amphibians, a thermogenic protein that has risen as the principal molecular thermogenic mechanism in mammals.

Methods

Sequence homologs search and AA primary structure prediction.

Complete coding sequence of known UCP1 of *Homo sapiens* and *Xenopus laevis* were retrieved from the GenBank (https://www.ncbi.nlm.nih.gov/). Then for research of

unknown amphibians and vertebrate reference UCP genes, we used the GenBank Blastn prediction tool. We extracted the whole amphibian transcriptome shotgun (TSA) and representative vertebrate genes (See Table S1 for their accession number). Then, we performed a conversion to amino acid sequences of the TSA using the Translate Tool of the ExPASy: SIB bioinformatics resource portal (https://www.expasy.org/). Duplicated sequences were eliminated manually from the full list obtained. Amino acid sequences from representative vertebrates were extracted using Protein blast and Homo sapiens UCP1 as reference. The UCP4 amino acid sequence from *X. laevis* were obtained from UniProt (see Table S2 for accession number) (https://www.uniprot.org/).

Comparative sequence analysis, aligments and phylogenetic analysis.

The DNA sequences were aligned using CLUSTALW algorithm (Dereeper et al., 2008; Larkin et al., 2007). Amino acid sequences were aligned using MUSCLE, and gaps were eliminated using Gblocks (Castresana, n.d.; Dereeper et al., 2008; Edgar, Drive, & Valley, 2004). Phylogenetic trees were reconstructed with PhylML using JTT+G+I as model for aminoacid sequences, GTR+G+ for DNA with 500 bootstraps in both cases (Dereeper et al., 2008; Lethiec, Duroux, & Gascuel, 2005). Trees were drawn using Figtree software (http://tree.bio.ed.ac.uk/software/figtree/). Invariant, conservative, parsimony and singleton sites were measured using Mega X software (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Sequence alignments and common secondary structure were visualized using MegAling Pro (https://www.dnastar.com/).

Computational Functional and structural Properties Discovery Pipeline.

Sequence logo maps of amino acid composition were measured by Web Logo online software (Crooks, Hon, Chandonia, & Brenner, 2004). Transmembrane structure of the amphibian UCP were made using Protter online server (Omasits, Ahrens, Mu, & Wollscheid, 2014). The homologous model of amphibian UCPs was build based on the structure of UCP2 from Mus musculus (BMRB: 17614), using the Protein Homology/analogy Recognition Engine PHYRE2 and I-TASSER (Kelley, Mezulis, Yates, Wass, & Sternberg, 2015). Models then were visualized, and residues tagged using Protter for membrane topology and VMD software for tertiary structures (Humphrey, William; Dalke, Andrew; Schulten, 1996; Omasits et al., 2014). Finally, we used DALI Protein Structure Comparison Server to perform an all against all structure comparison obtaining a structure similarity dendrogram (Holm & Laakso, 2016).

Results

Amino acid sequence prediction.

The increasing number of molecular repertories described in recent years allows the re-understanding of the family status of proteins as in UCP family. New approaches based on molecular phylogeny, structure prediction and comparison with focus on the functional structure, allow us to understand more deeply the adaptational functional structure of amphibian UCPs. We predicted 100 protein encoding genes (Table 1) of unidentified amphibian UCPs with informative E-Values (<2e-172) and identity percentage (<80% for most cases). Then, following the conversion from nucleotide to amino acid sequences, alignments and phylogenetic analysis, cleansing of duplicates and incomplete sequences, we obtained the final list of members of the amphibian uncoupling

proteins (Table 1). 62 % of the functional proteins were found at the open reading frame 1 (ORF), 21% in ORF2 and 17% in ORF3. The functional sequences found have an average of 309 amino acids.

Comparative analysis and phylogenetic analysis.

The cleaned alignments obtained were used to construct phylogenetic trees with the maximum likelihood (ML) method. Analyses were based on a sequence data that include 100 DNA sequences and amino acid sequences obtained from amphibian UCPs. For DNA we used a total of 108 reference UCPs genes from mammalian, reptile and fish. The ML analysis of those sequence data set using a midpoint rooting confirmed that members of the vertebrate UCP family are grouped on 3 main clades: UCP1, UCP2 and UCP3. Each of the clusters showed a high bootstrap support, except for the UCP3 members. As expected inside each paralog cluster, amphibian UCPs orthologs were recovered at the basal position with respect of mammals and reptiles. Reflecting phylogeny of taxa analyzed (Figure 1 and Figure 2). Interestingly, the most intriguing feature of the recovery trees was the long branch from the common ancestor of the UCP1 to the mammalian orthologues, what strongly suggests selection during UCP1 evolution. Furthermore, here we extent the existence of the amphibian clade. Which recovers the UCP1 genes as basal group according to the trees, a fact previously unreported.

Once amphibian UCPs amino acid sequences were characterized, a phylogenetic analysis of the UCP gene family inside this taxon were performed. For these, a ML tree was reconstructed showing strong support for the 3 UCPs clades. As expected, UCP paralogs were shown as a history of duplication as in other taxons like in mammalian gene history. In contrast, ortholog diversification indicates different selection and

diversification processes. For UCP1, the basal ancestral state was occupied by the anuran ortholog, being the sister clade of salamandrian and gymnophionan copies. In UCP2 and 3 we found that the ancestral state was occupied by the gymnophionan ortholog, having as sister group the salamandrian and anuran copies. For all paralogues cases, internal nodes present a monophyletic state, as the total reconstructed tree of the protein family. Moreover, we found peculiarities for some species inside the major amphibian clades. One of these events was the strong selection that occurred during UCPs evolutionary history and adaptation. First, inside the UCP1 paralog, the Gymnophiona orthologues present a longer branch from the common ancestor of salamandrian copies, especially the specie *Rhinatrema bivittatum*, the one presenting the longest branch inside the entire UCP tree. Second, the anuran *Rhinella marina* was found as the second specie with the longer branch inside the entire UCP family tree. Finally, no strong selection was found inside the UCP2 cluster (Figure 3).

Structural and functional properties.

Multiple sequence alignment of the different members from amphibian UCP1, allow identification of the amino acid residues that are conserved and shared with mammalian UCP1 sequence within and between those groups (Figure 4). Phylogenetic analyses were based on the amphibian UCP1 orthologues that included 310 positions, of which 160 were variable, 150 conserved, 107 parsimony informative and 53 singletons. Amphibian UCP1 has tree domains of 100 amino acids. Each one possesses one mitochondrial carrier signature (Figure 4) that is well conserved in other mitochondrial carriers. A nucleotide binding signal sequences were also found (Figure 4). Conservative and shared functional residues of amphibian and mammalian sequences (Figure 4 and Figure 7) locating indispensable residues are shared with mammalian functional protein.

Moreover, as illustrated, using HMM logos (Schuster-böckler, Schultz, & Rahmann, 2004) for amphibian UCP1, great part of this residues are found as the main probability sequence with the functional residues (Figure 5). We take the one previously used for the final consensus sequence manual to optimize the prediction. The structural transmembrane topology revealed the conservative structure of three repetitive domains, 6 transmembrane helixes, the ones that are connected by 5 loops and the N-termini as well as the Carboxyl termini protruding into the intermembrane space (Figure 6, Figure 7 and Figure 8). The comparison between other mammalian UCP1 model structures revealed that the proteins found in the two clades were clustered structurally among them, finding amphibian proteins very closely related to their functional homolog in mammals (Figure 9). Also, functional residues were tagged and compared with the mammalian Thermogenin, revealing a high conservation of topological ubication of these residues (Figure 10 and Figure 11).

Discussion

The present study, focuses on expanding previous molecular findings regarding UCP1 evolution with an important emphasis on amphibian orthologs. By an extenuate research of amphibian coding UCP sequences from transcriptome data base, we demonstrate the close relationship between the functional UCP1 of mammals and their homologue in amphibians; allowing us to predict a preserved function guided by its sequence and structure. This work is the first to analyze in depth the molecular structure and functional residues of the amphibian UCP1. The phylogenetic tree reconstruction of DNA sequences recoils well stablished topologies with good support values for basal relationships than using the data provided by amino acid sequences, as a result of the

highly conserved protein sequences. Moreover, in vertebrate reconstruction as in amphibian one, phylogeny of UCP indicates that UCP1 is the ancestral UCP, which is present in other species as previously demonstrated (Hanák & Jezek, 2001; Hughes & Criscuolo, 2008; Jiménez-Jiménez et al., 2006; Keller et al., 2005; Ledesma et al., 2002; Saito et al., 2008); then, the UCP1 doublet prior divergence of vertebrates (Hughes & Criscuolo, 2008). Later, a second duplication occurs, allowing the divergence of UCP2 and UCP3 (Figure 1. and Figure 2.). Here we reconstruct the phylogenetic relationships of UCP family evolution in the three amphibian main clades: Gymnophiona, Salientia and Caudata. We found a history of duplications of the protein family as a hallmark in amphibian UCP evolution (Figure 3.). Interestingly, inside the UCP1 amphibian orthologs, the aquatic specie Rhinatrema bivittatum is suggested as the ortholog member with the strongest selection support. This species of caecilian belongs to the most ancestral group of Gympnophionans, the Rhinatrematids (Mark, n.d.). R. bivitattatum have a free-living stage larvae, resisting adverse conditions during its development. This stage has characteristics with more resemblance to ancestral states (Michigan, 1968). It has been proposed for UCP evolution, that the activity of this protein during embryological development and growth takes a crucial role on the energy balance and thermogenesis among vertebrates especially in mammals (Echtay et al., 2018; Saito et al., 2008). This would support the UCP1 selection observed within this group. According with this idea, the developmental process found on the caecilians, such as viviparous and oviparous life forms, could support the strong selection of this paralogue. Is also notorious the strong selection upon Typhlonectes compressicauda, the one that present viviparity and a well develop respiratory system (McKitrick, 1993; Wilkinson & Nussbaum, 1998). UCP1 could have faced a variation achieved during the evolution of this clade, which is supported by changes on the reproductive forms and systems

dependent of energy transfer mechanisms observed in the mitochondria. Besides the early divergence of amphibians, this strong selection of Gymnophionan, could have been caused by an unreported specialized activity that could be study more deeply in the future (Rey et al., 2008; Schwartz, Murray, & Seebacher, 2008; Vianna et al., 2017).

Early studies demonstrated the occurrence of UCP in a great number of clades among eukaryotes, indicating the conservation of many important residues among the UCPs sequences (Boss, Muzzin, & Giacobino, 1998; Keller et al., 2005; Ledesma et al., 2002). Here, we observed a conserved structure of the amphibian UCP 1, based on functional topologies, sequences signatures and amino acids residues previously reported and related to the functionality of this protein (Figure 4 - 9) (Keller et al., 2005). In the amino acid alignment in Figure 4 and Figure 5, as well as in models obtained in Figure 10, three of the four amino acids present in mammals for the proton translocation pathway were found in amphibians UCP1. The comparison between mammalian and amphibian structures, showed that residues form the translocation pathway for this mechanism is conserved in both cases. This mechanism works when the carboxyl group of the Histidine (145/147) or Asparagine (8/28) protrude inside the channel, bind to the protons and with the help of the fatty acids that bind into their structure, translocate those ions from the intermembrane space to the matrix (Crichton, Lee, & Kunji, 2017; Echtay et al., 2018; Jastroch, Withers, & Klingenspor, 2004; Jiménez-Jiménez et al., 2006; Suzuki, Murata, & Oda, 2006; VIANNA et al., 2017). Moreover, the missing amino acid in the amphibian sequence has been previously reported as an unnecessary for its uncoupling activity (Echtay et al., 2018; Jiménez-Jiménez et al., 2006). For residues involved in nucleotide binding, we found that all of the AA were present in the gate mechanism, the phosphates binding and the pH sensor (Figure 4, Figure 7 and Figure 11), except for an accessory residue involved in the last inside mammals. Consequently, Glutamine 190 and Arginine

95 normally bind each other promoting a physical block. Also, the pH sensor residues, Histidine 214 and Asparagine 209/210 constantly have a free stage. Moreover, when pH becomes more acidic, Glutamine 190 and Histidine 214 protonate leading to a physical change in the protein. First, the gate goes from a close to an open state by blocking the bond of Glutamine and Arginine. Second, the pH sensor goes to a binding form, promoting the movement of the second intermembrane space loop which is normally found blocking the channel. In consequence, the Arginine 83/182/276 are exposed and a nucleotide (GDP/GTP/ADP/ATP) can bind blocking the proton translocation pathway (F. Bouillaud et al., 2018; Crichton et al., 2017; Echtay et al., 2018; Jastroch et al., 2004; Ježek & Garlid, 1998; Jiménez-Jiménez et al., 2006; Klingenberg, 1993; Mao et al., 1999; Rey et al., 2008; Rial, González-Barroso, Fleury, & Bouillaud, 1998). Finally, other residues involved in post transcriptional modification were found (Figure 7) (Carroll, Porter, & Morrice, 2008; Echtay et al., 2018).

Conclusion

The use of bioinformatic tools as well as genomic data have provided a deeper understanding of the evolution of UCPs in the amphibian clade, as well as the structural functionality of the UCP1. Here we evidence an ecological selection based on the reproductivity mode that differs among amphibians. We obtain the model structure of the amphibian Thermogenin demonstrating the conservation of functional residues leading to the acceptance of functionality by structure prediction. Future studies involving the activation of mediators as fatty acids or PPARy should be performed in a future to better understand the UCP role in thermogenesis. Understanding the molecular adaptations of amphibians to low temperatures is key to comprehend the eco-physiological distribution

of this clade worldwide. Our study provides information in a field that has been neglected however important to comprehend how amphibians survive and will persist in the future.

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Apendix

Figures

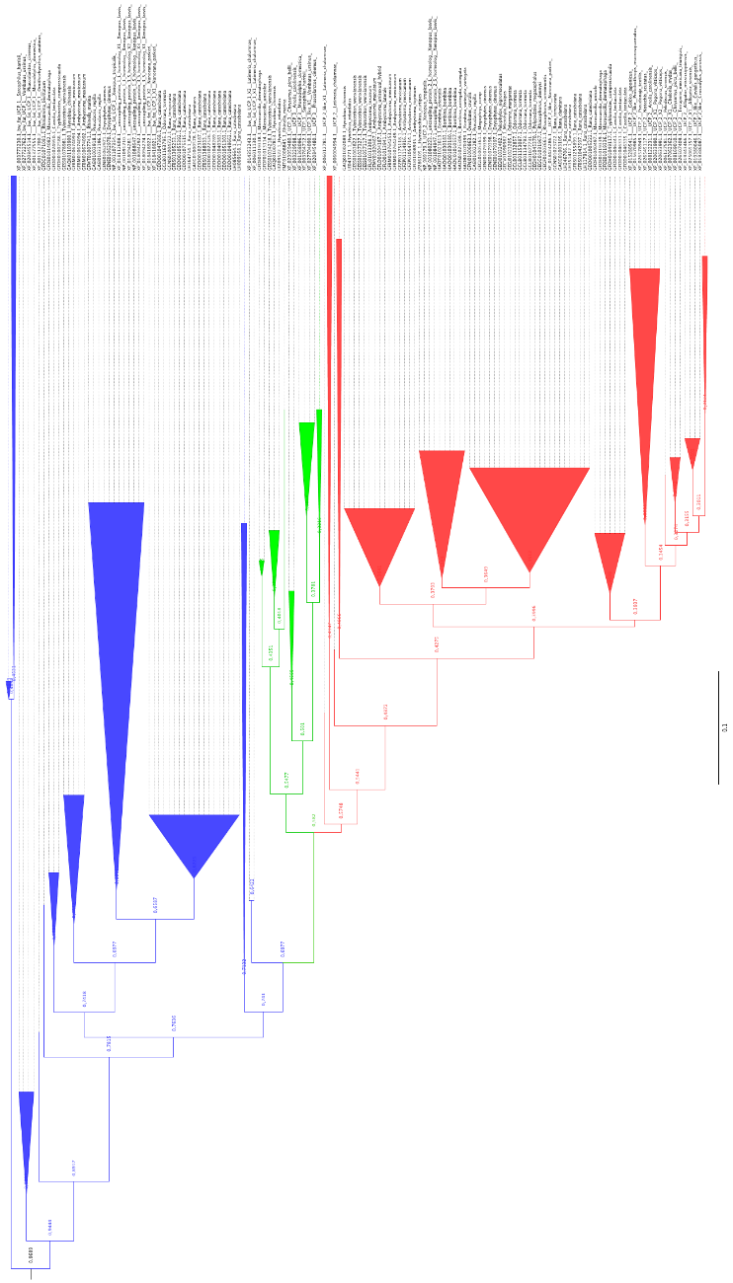


Figure 1: Phylogenetic relationship among the UCP family of vertebrates. A DNA phylogenetic tree was constructed using Maximum likelihood and midpoint rooting. The reconstructed tree was used to cluster unidentified amphibian UCP sequences extracted from Gene Bank. Black lines represent ancestral UCP, blue lines UCP1, green lines UCP3 and red UCP2.

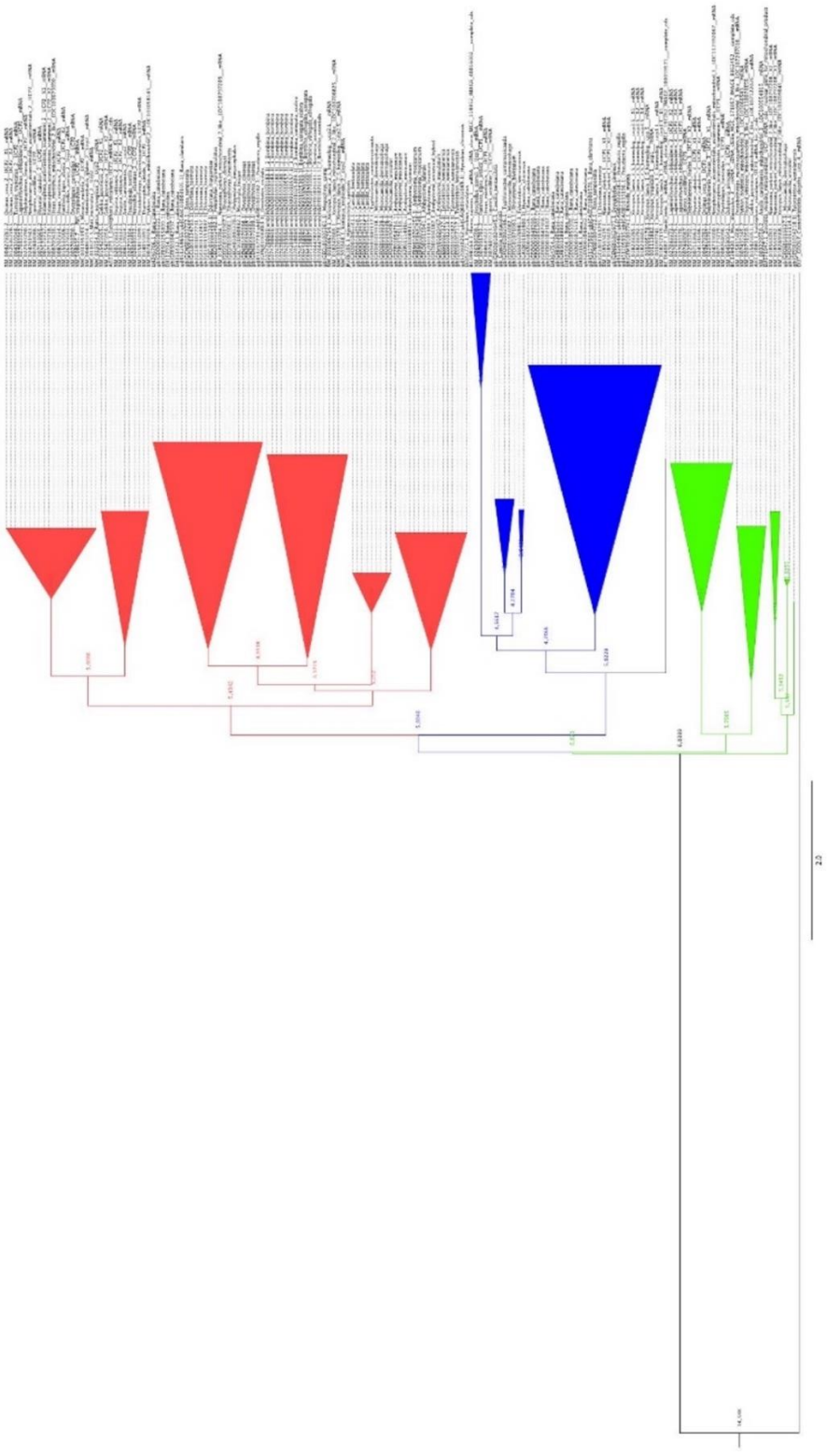


Figure 2: Amino acid phylogenetic relationship among the UCP family of vertebrates. A amino acid phylogenetic tree was constructed using Maximum likelihood and midpoint rooting. The reconstructed tree was used to cluster unidentified amphibian UCP sequences extracted from Gene Bank and transform into AA sequences. Black lines represent ancestral UCP, blue lines UCP1, green lines UCP3 and red UCP2.

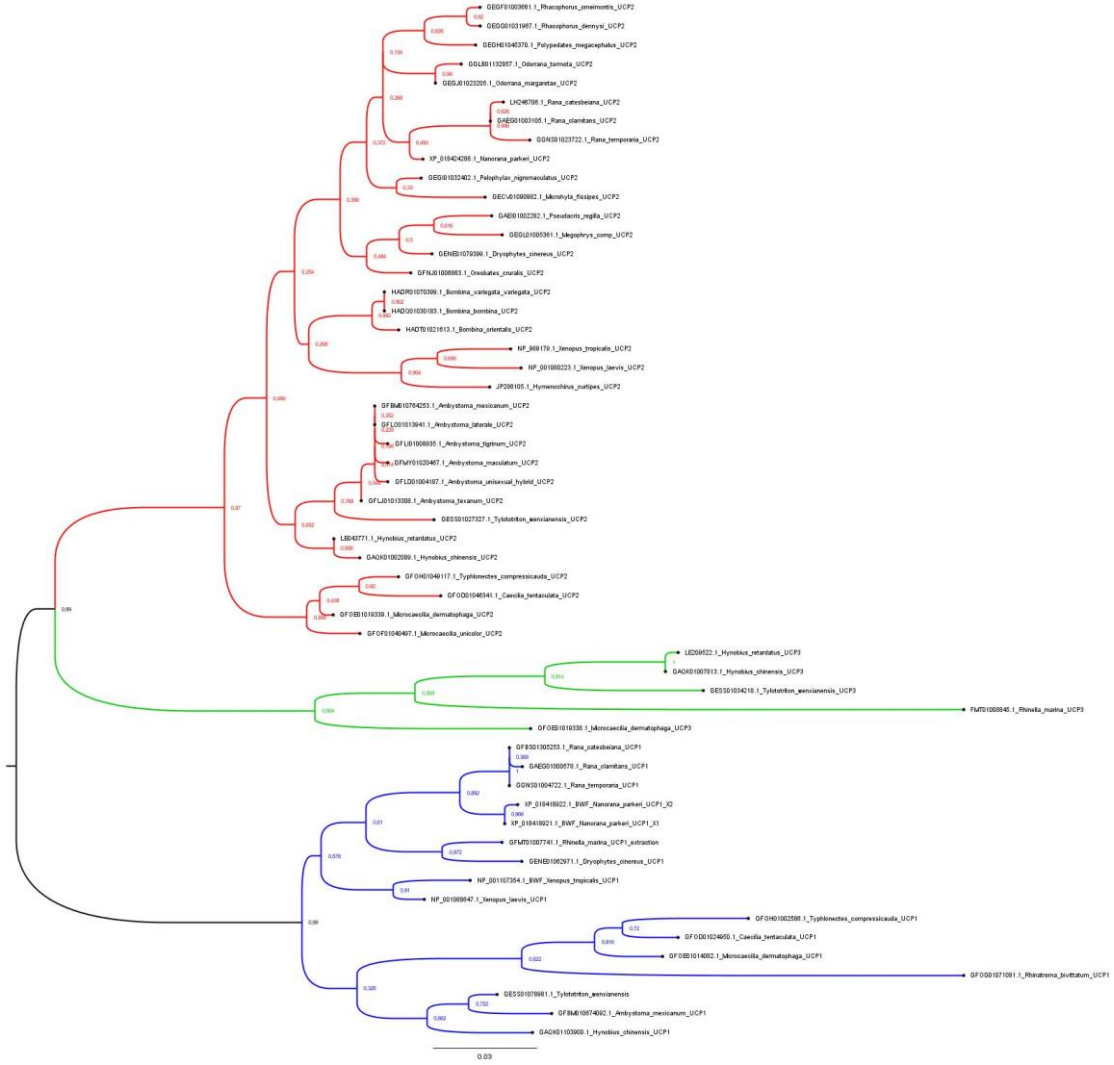


Figure 3: Amino acid phylogenetic relationship among the identify amphibian UCP family . A amino acid phylogenetic tree was constructed using Maximum likelihood and midpoint rooting. A 500 bootstrap were used as statistical tests and a JTT+G+I as model of evolution. Black lines represent ancestral UCP, blue lines UCP1, green lines UCP3 and red UCP2.

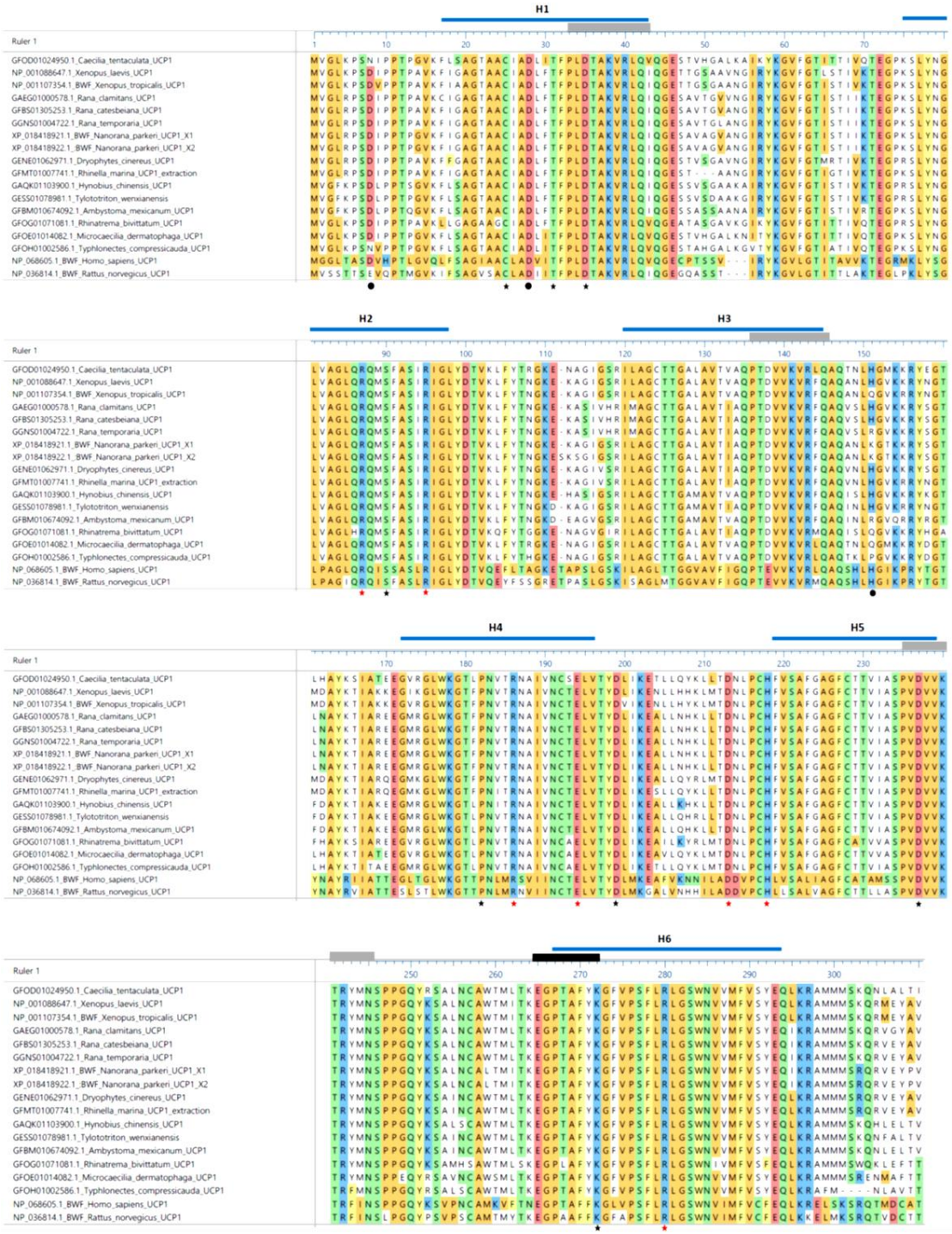


Figure 4: Alignment of the human (NP_068605.1), Nordic rat (NP_036814.1), and amphibians UCP1 obtained with Mega Alig. The sequences are represented in single letter code. Gaps introduced to optimized sequences are represented by a dash. Identical amino acid mapped to human UCP1 are highlighted in biochemical color code. Grey boxes represent mitochondrial carrier family signal. Black box highlights the Nucleotide binding signal present in all members of the super family. Marks bellow represent important amino acids for UCP function: Black dots indicated residues important for

H+ transport, red starts for nucleotide binding mechanism and black stars other important functional amino acids.

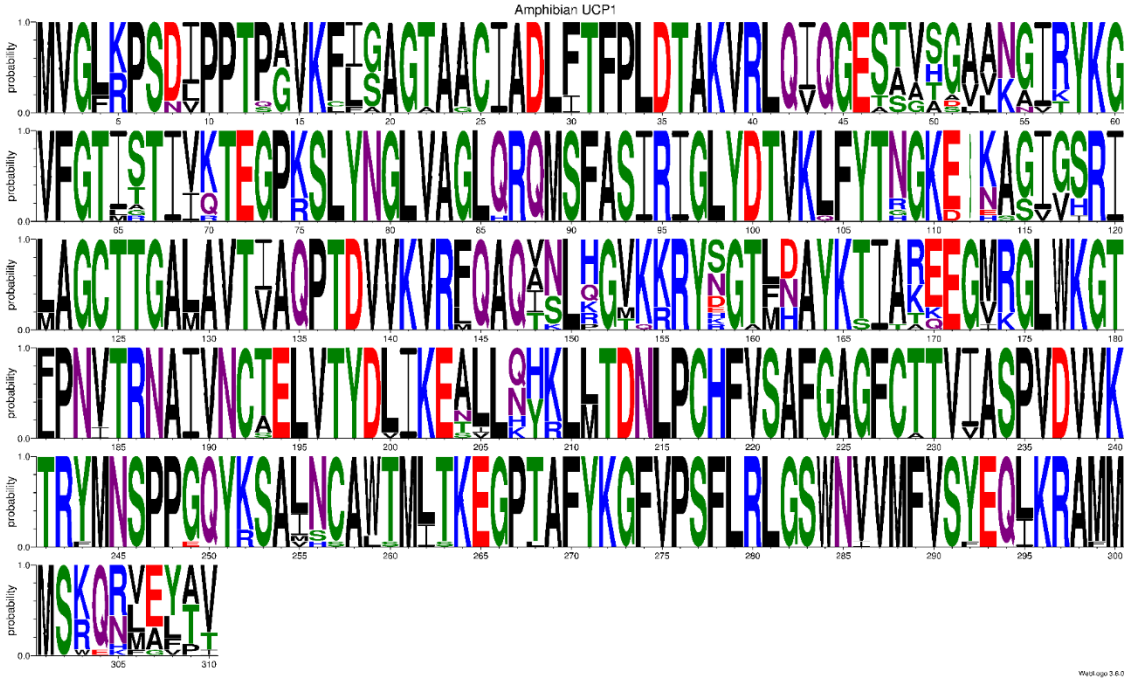


Figure 5: HMM logos for amphibian UCP1. Variety, conservancy and shared residues inside the amphibian UCP1 sequence. Important aminoacids are the most shared between sequences. Position S112 its only found in one sequence.

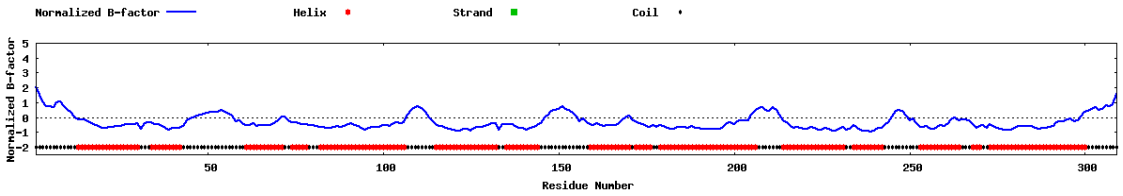
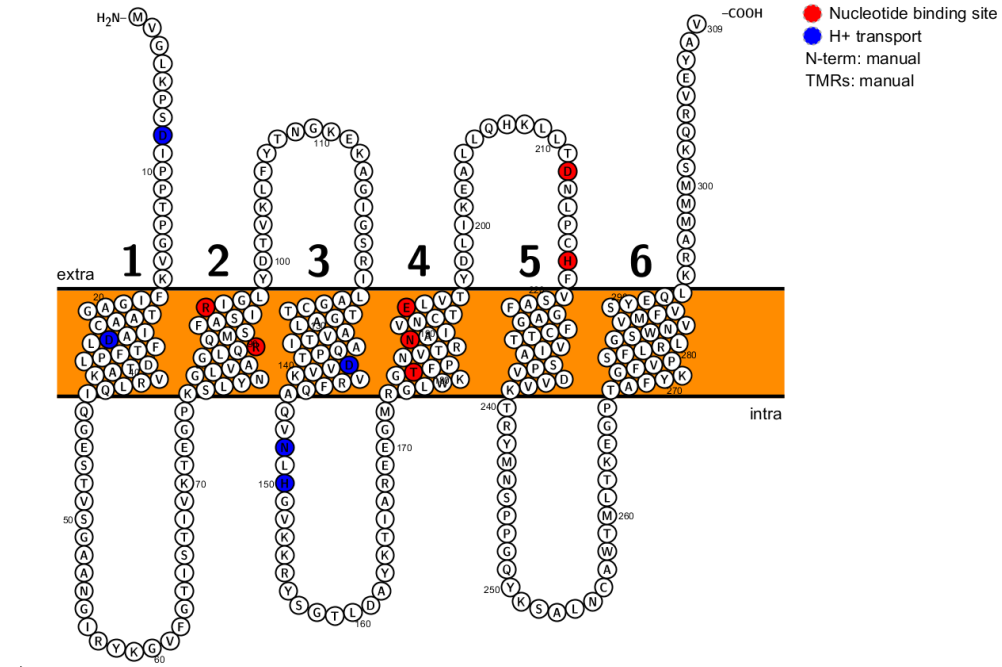
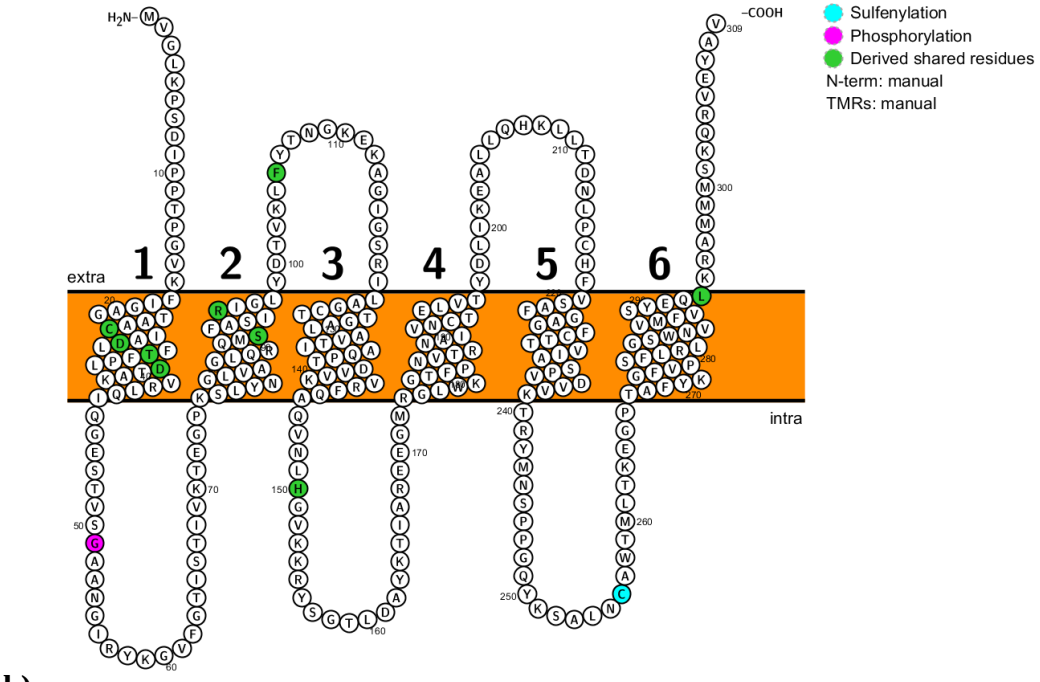


Figure 6: Hydrophobicity plot of consensus amphibian UCP1. The normalize B-factor give us the hydropathy behavior of the molecule. Red Asterix represent helix and black dots coils inside the sequence.



a)



b)

Figure 7: Transmembrane secondary structure of consensus amphibian UCP1. a) Shared and functionals residues for nucleotide binding and H+ translocation. b)

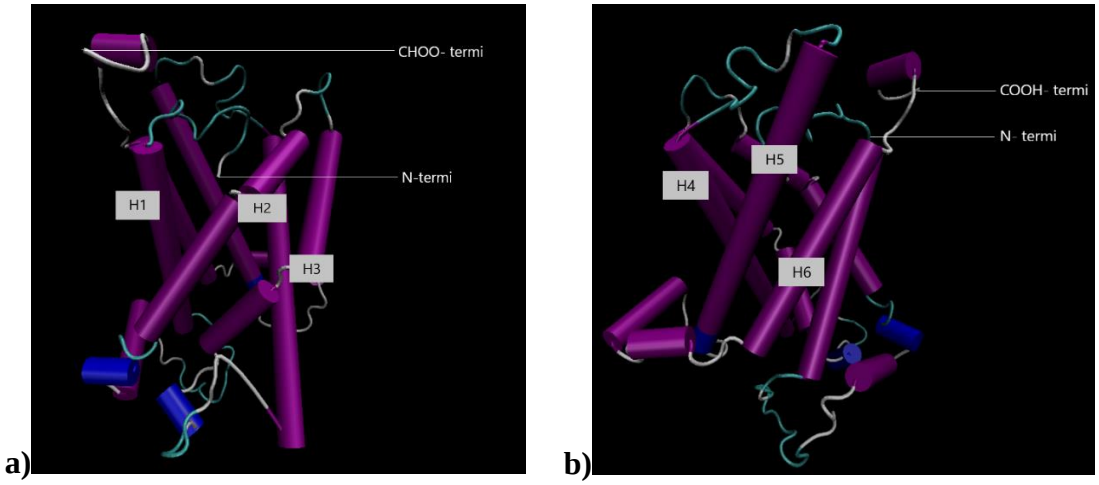


Figure 8: Molecular tertiary structure of consensus amphibian UCP1. Cartoon representation acquire by VMD software. A tripartite structure is represented. N and carboxyl termi both protruding to the intermembrane space. a) transmembrane helix 1 - 3. b) transmembrane helix 4 – 6.

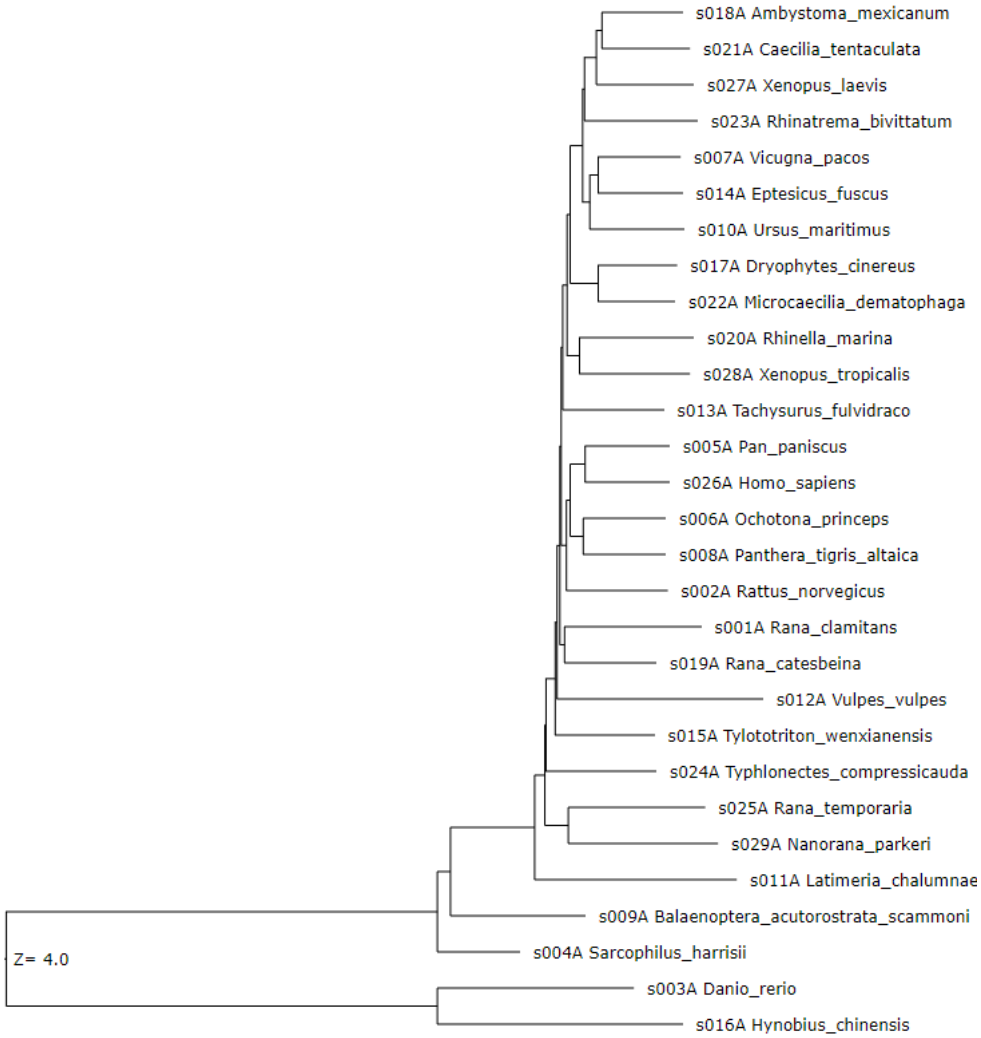
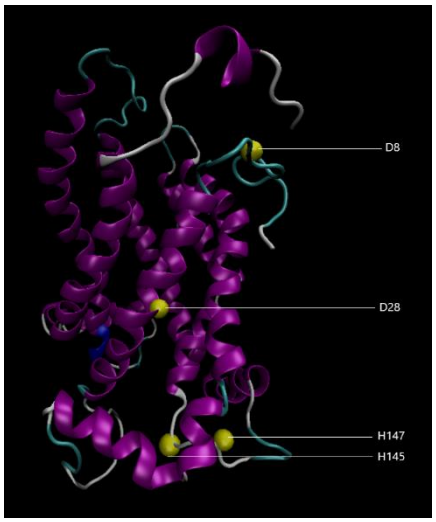
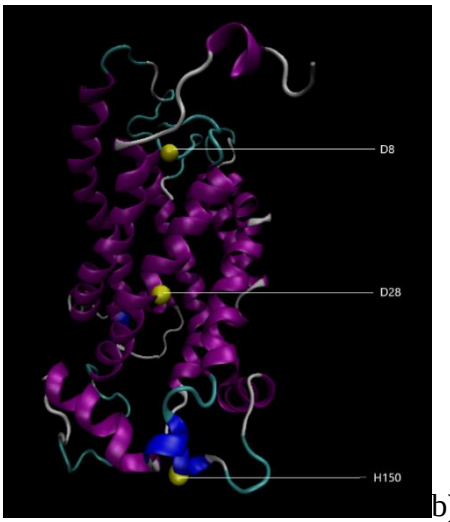


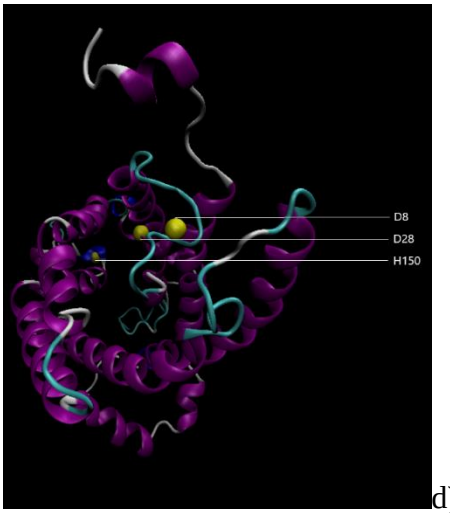
Figure 9: Structural relationship dendrogram of amphibian vs. vertebrate UCP1. Structural relationships tree was constructed using Dali server. Red dot indicates the point were functional structures clots.

a) 



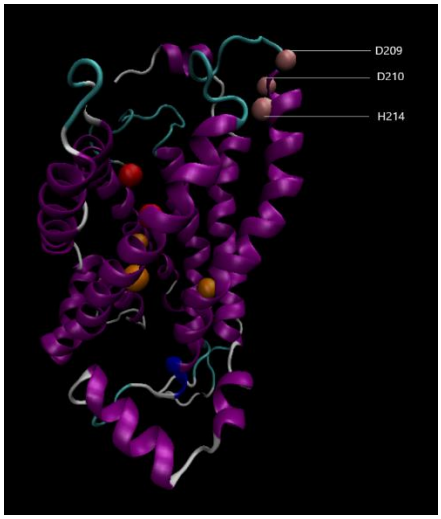
b)

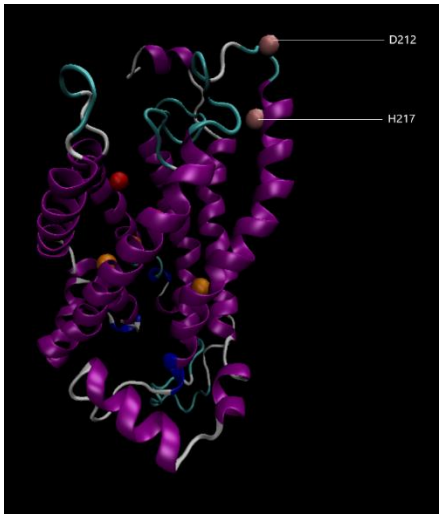
c) 



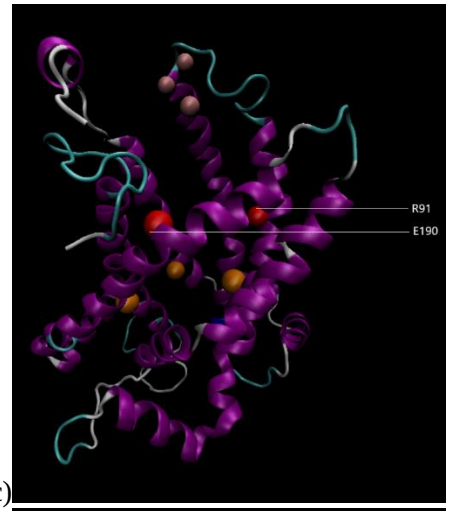
d)

Figure 10: Molecular mapping and comparison of residues involve on proton translocation pathway between amphibian and human UCP1. Left human, right amphibian UCP1. Localization of important residues were tagged with yellow spheres. a) and b) frontal view of trans locational pathway. c) and d) axial view from the intermembrane space on front to the matrix side in the back. Here you can note the channel trough the carrier.

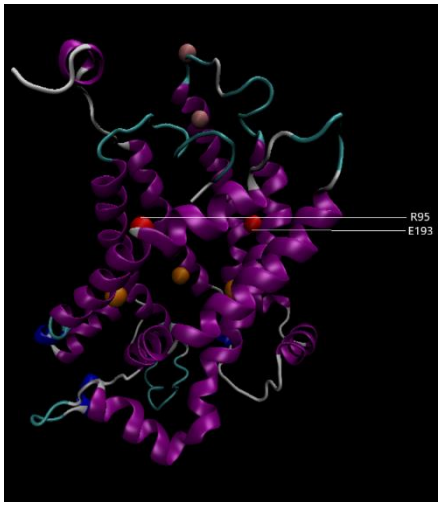
a) 



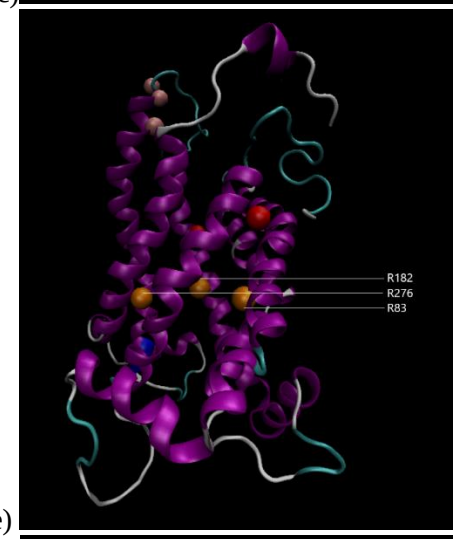
b)


R91
E190

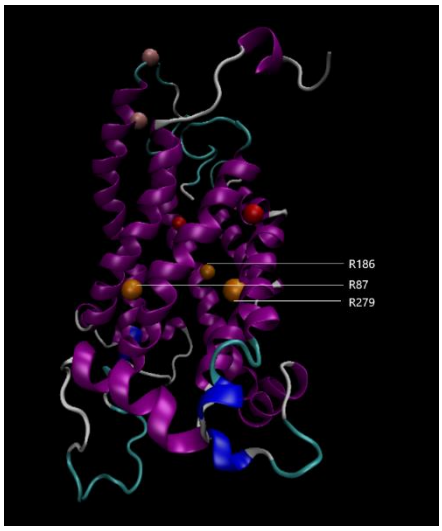
c)


R95
E193

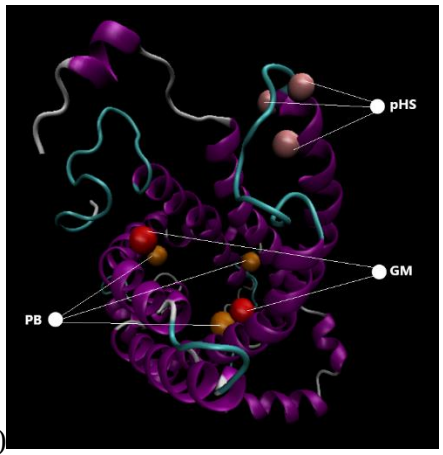
d)


R182
R276
R83

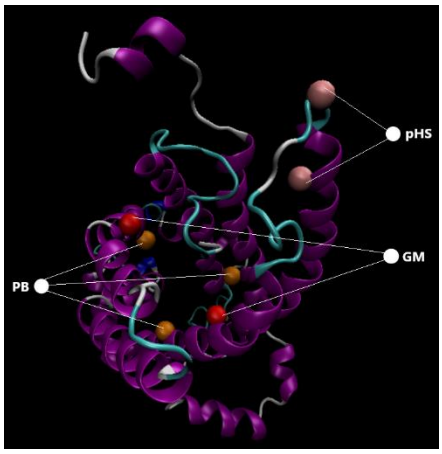
e)


R186
R87
R279

f)


pHS
GM
PB

g)


pHS
GM
PB

h)

Figure 11: Molecular mapping and comparison of nucleotide binding residues between amphibian and human UCP1. Structures: left human, right amphibian UCP1. Localization of important residues were tagged with spheres. a) and b) frontal view of the pH sensor residues in pink (pHS). c) and d) frontal view of the gate mechanism (GM). e) and f) frontal view of the phosphates binding site (PB). g) and h) axial view of the nucleotide bindind residues neighborhood, from the intermembrane space on front to the matrix side in the back. Here you can note the channel trough the carrier.

Tables

Table 1: Predicted amino acid sequences of TSA UCPs used for Amphibian reconstruction

ACC NUM	SPECIE	Frame	#AA
GFLO01013941.1	*Ambystoma laterale*	5'3'F1	308
GFMY01020467.1	*Ambystoma maculatum*	5'3'F2	308
GFBM010674092.1	*Ambystoma mexicanum*	5'3'F3	309
GFBM010764253.1	*Ambystoma mexicanum*	5'3'F1	308
GFLJ01013308.1	*Ambystoma texanum*	5'3'F1	308
GFLI01008935.1	*Ambystoma tigrinum*	5'3'F1	308
GFLD01004187.1	*Ambystoma unisexual hybrid*	5'3'F1	308
HADQ01030183.1	*Bombina bombina*	5'3'F1	308
HADT01021613.1	*Bombina orientalis*	3'5'F1	308
HADR01070399.1	*Bombina variegata variegata*	5'3'F2	308
GFOD01046341.1	*Caecilia tentaculata*	5'3'F1	309
GFOD01024950.1	*Caecilia tentaculata*	5'3'F1	309
GENE01062971.1	*Dryophytes cinereus*	3'5'F1	309
GENE01079399.1	*Dryophytes cinereus*	3'5'F3	308
JP286105.1	*Hymenochirus curtipes*	5'3'F1	292
GAQK01007813.1	*Hynobius chinensis*	3'5'F3	309
GAQK01103900.1	*Hynobius chinensis*	3'5'F2	309
GAQK01002089.1	*Hynobius chinensis*	5'3'f3	309
LE209522.1	*Hynobius retardatus*	3'5'F2	309
LE043771.1	*Hynobius retardatus*	5'3'F3	310
GEGL01005361.1	*Megophrys comp*	5'3'F1	308
GFOE01019338.1	*Microcaecilia dermatophaga*	5'3'F1	309

GFOE01014082.1	*Microcaecilia dermatophaga*	5'3'F1	309
GFOE01019339.1	*Microcaecilia dermatophaga*	5'3'F1	308
GFOF01040497.1	*Microcaecilia unicolo*	5'3'F1	309
GECV01090982.1	*Microhyla fissipes*	5'3'F2	308
GEGJ01023205.1	*Odorrana margaretae*	3'5'F3	308
GGLB01132857.1	*Odorrana tormota*	3'5'F3	308
GFNJ01006863.1	*Oreobates cruralis*	5'3'F2	308
GEGI01032402.1	*Pelophylax nigromaculatus*	3'5'F1	308
GEGH01045370.1	*Polypedates megacephalus*	3'5'F1	308
GAEI01002282.1	*Pseudacris regilla*	5'3'F1	308
GFBS01305253.1	*Rana catesbeiana*	5'3'F1	309
LH246706.1	*Rana catesbeiana*	5'3'F1	308
GAEG01000578.1	*Rana clamitans*	5'3'F2	309
GAEG01003105.1	*Rana clamitans*	5'3'F1	308
GGNS01023722.1	*Rana temporaria*	5'3'F2	308
GGNS01004722.1	*Rana temporaria*	5'3'F2	309
GEGG01031967.1	*Rhacophorus dennysi*	3'5'F1	308
GEGF01003661.1	*Rhacophorus omeimontis*	5'3'F1	308
GFOG01071081.1	*Rhinatrema bivittatum*	5'3'F1	309
GFMT01008845.1	*Rhinella marina*	5'3'F3	309
GFMT01007741.1|	*Rhinella marina*	5'3'F2	346
GESS01027327.1	*Tylototriton wenxianensis*	5'3'F1	310
GESS01078981.1|	*Tylototriton wenxianensis*	5'3'F1	309
GESS01034218.1	*Tylototriton wenxianensis*	5'3'F1	303
GFOH01049117.1	*Typhlonectes compressicauda*	5'3'F1	309
GFOH01002586.1	*Typhlonectes compressicauda*	5'3'F1	305