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Review Article

Structural-Functional Characterization of Cytochrome b in bc_1 and b_6f Complexes along with Polymorphic Analysis

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ABSTRACT

Mitochondrial cytochrome b (cyt b) transfers the electrons in bc_1 complex from ubiquinone to cytochrome c reductase in the mitochondrial respiratory chain, and similarly in b_6f enzyme complex, from plastoquinol (QH_2) to plastocyanin (Pc) within the thylakoid membrane. Mitochondrial cyt b in bc_1 complexes contains eight transmembrane helices A to H; two of these, B and E along with two histidines, are cross-linked with two hemes located at the top and bottom of the membrane. The b_6f complex contains four large subunits which include cytochrome f, b, Rieske iron-sulfur protein, and subunit IV. Electrons transferred through these complexes are responsible for the pumping of protons across the membrane to produce ATP through ATP synthase. The present review provides the structural comparison of mitochondrial cyt b in ten model organisms targeting archaea, prokaryotes, and eukaryotes, highlighting phylogenetic and mutational analysis. Polymorphism in the mitochondrial cyt b gene helps in studying the biodiversity and is a valuable tool for the identification of species. Mutations in the cyt b gene produce abnormal protein leading to deficiencies in the complex III (coenzyme Q), resulting in defective oxidative phosphorylation and consequently effects other metabolic pathways. In the present article, we comprehensively compare the biodiversity of cyt b in bc_1 and b_6f complexes using in silico structural analysis tools, emphasizing that despite vast knowledge available in this field, still there are so much to explore about cyt b.

INTRODUCTION

Cytochrome b protein has a comprehensive role in the transportation of electrons in the respiratory chain of mitochondria. Cytochrome b is encoded by a single gene in the mitochondria, whereas in the case of chloroplast two genes are involved for the synthesis of this protein (Dumas *et al*., 2018). This polypeptide chain has a central role as electron transport in the bc_1 and b_6f transmembrane cytochrome complexes. Mitochondrial cytochrome b as a part of the bc_1 complex transfers the electron from ubiquinone (QH_2) to the cytochrome c reductase in the mitochondrial respiratory chain (Seddigh and Darabi, 2018). Four proton molecules ($4H^+_P$) are accumulated at the positive side of the lipid bilayer membrane by the oxidation of every ubiquinone (QH_2) along with the reduction of two cyt c ($2c^{3+}$) molecules (Equation 1). Mitochondrial cytochrome b present in the respiratory chain and chloroplast plastoquinol acceptor reductase (EC 7.1.1.6) are homologous in their function (Bhaduri *et al*., 2019). This chloroplast enzyme (b_6f complexes) has a significant role in photosynthesis by transferring the electron from photosystem-II (PSII) to photosystem-I (PS-I). Moreover, this chloroplast enzyme contains two b and two c type cytochromes. The coenzyme Q also known as complex III is encoded by cytochrome b has a fundamental role in the production of ATP during oxidative phosphorylation (Stefely and Pagliarini, 2017).

$$QH_2 + 2c^{3+} + 2H^+_N \xrightarrow{bc1} Q + 2c^{2+} + 4H^+_P \quad \ldots(1)$$

Energy is required for the survival of all the organisms on this planet. All organisms possessing mitochondria except protozoa like *Trychomonas* contain cytochrome b and photo-redox proteins, which have a central role in ATP generation (Ramsay, 2019). ATP is renowned

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as a cellular currency for all living organisms provides energy to all the metabolic processes occurring in living cells. Some homologous proteins were also reported in the plant chloroplasts and cyanobacteria (cytochrome b_6) that contribute their role in the b_6f complex also known as plastoquinone–plastocyanin reductase complexes (EC 1.10.99.1) (Strand *et al*., 2017). The electrons transferred through these complexes are responsible for the pumping of protons across the membrane, produce ATP through membrane-bounded ATP synthase (Mansilla *et al*., 2018). ATP synthase works like a turbine, which is driven by the flow of protons from higher concentration to lower concentration across the membrane. ATP is generated from ADP by the addition of phosphate with ATP synthase which becomes active by proton motive force. ATP production mechanism in the electron transport chain located at the inner surface of the mitochondrial membrane is explained in Figure 1 (Nelson and Cox, 2017).

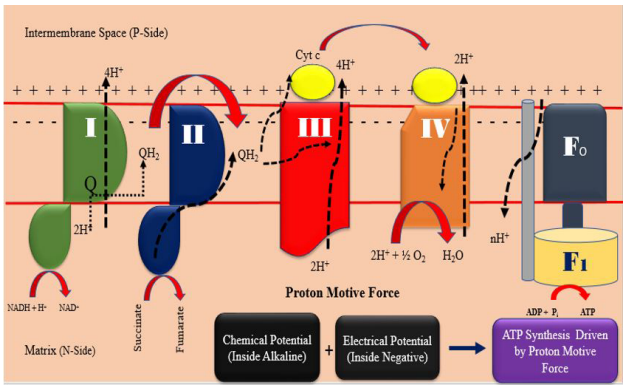

Fig. 1. ATP production mechanism at mitochondrial electron transport chain (drawn by the authors).

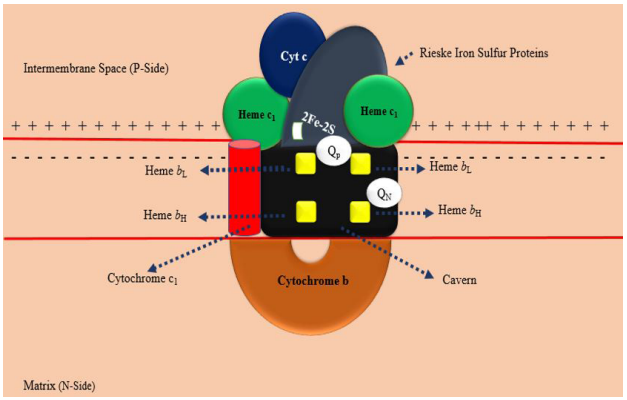

Fig. 2. Structure of mitochondrial cytochrome b in bc_1 complex (drawn by the authors).

Structure of cytochrome b in mitochondrial bc_1 *complex*

Cytochrome bc_1 belongs to one of the three respiratory enzymes complexes located at the inner membrane in mitochondria. This complex transfers the electrons from ubiquinone to the cytochrome c reductase in the mitochondrial respiratory chain and uses energy from the electrochemical gradient across the membrane (Seddigh and Darabi, 2018). Cytochrome b in the mitochondrial genome is encoded by a single gene *petB* whereas in the chloroplast, encoded by *petB* and *petD* genes. The *petD* gene has a fundamental role in the production of subunit IV of the cyt b_6f complex (ΔpetD) in the thylakoid membrane of the chloroplast (Dumas *et al*., 2018). The complete genome size of the mitochondrial cytochrome b gene is approximately 1140 bp (Lestari *et al*., 2018). The molecular mass of cytochrome b protein, along with hemes in the bc_1 complexes, is approximately 42.5 kDa as reported by (Berry *et al*., 2000). The ATP production mechanism in the electron transport chain located at the inner surface of the mitochondrial membrane is explained in Figure 2 (Nelson and Cox, 2017). Cytochrome b from bc_1 complex contains two ubiquinol/ubiquinone (Qo/Qi) and two hemes binding sites. Two hemes groups b562 and b566 are non-covalently attached to the cytochrome b in the mitochondria. Potentiometric titration showed that mitochondrial cytochrome b has two midpoint potential species included b_H and b_L. The species have higher midpoint potential represented by H while lower denoted by L. Two types of hemes b_L and b_H are present in the first helices bundles. Four histidine residues His84 and His183, His98 and His197 are present in the axial ligands of the b_L and b_H hemes and are highly conserved in the cytochrome b gene (Gao *et al*., 2003). The two species of hemes b_H and b_L have different properties and environments within the same protein.

The mitochondrial cytochrome b in bc_1 complexes contains eight transmembrane helices A, B, C, D, E, F, G, and H (Dumas *et al*., 2018). Two transmembrane helices B and E in mitochondrial cyt b along with two histidine molecules are crosslinked with two hemes located at the top and bottom of the membrane. The 3D structure of mitochondrial cytochrome b in *Mycobacterium tuberculosis* is elaborated in Figure 3. The secondary structures of the mitochondrial cytochrome b are labelled with a spectrum ribbon along with N- and C-termini. The eight transmembrane helices of cyt b are labelled as A to H red alphabets (Fig. 3). The C and N termini of the mitochondrial cyt b are in the matrix region (Ko and Choi, 2016). The first five helices, A to E is surrounded by two hemes b_H and b_L subunits, are called first bundles. Q_N inhibitor site is present in the first bundle near to b_H hemes site of the cyt b. The remaining three helices F, G, and H

compose the second bundle of the cyt b. Q_P site is located near to the b_L hemes is composed of helices C and F (Ko and Choi, 2016). The lipid bilayer of the mitochondrial membrane is represented as a doted red line (Fig. 3).

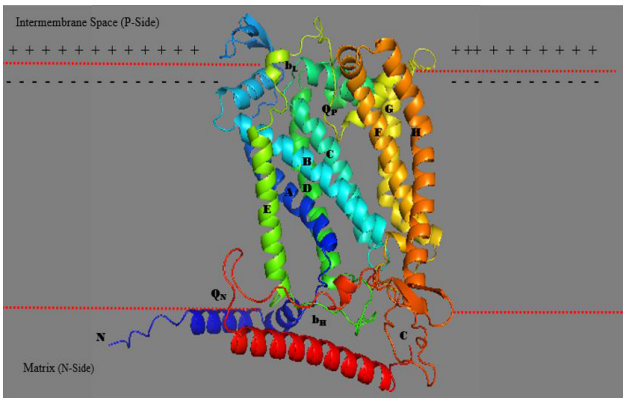

Fig. 3. 3D structure of mitochondrial cytochrome b in *Mycobacterium tuberculosis* bc_1 complex (drawn by the authors).

Structure of cytochrome b in chloroplast b_6f *complex*

Cytochrome b_6f complex exists as a dimer in the thylakoid membrane of the chloroplast. This complex contains four large subunits which included cytochrome f, cytochrome b, Rieske iron-sulfur protein, and subunit IV. Chloroplast b_6f complex contains seven transmembrane helices in which the first four transmembrane helices A, B, C, and D are present in cytochrome b subunit of the b_6f complex whereas subunit IV of the complex contains E, F, and G helices (Dumas *et al*., 2018). Different *pet* gene series is involved to produce the different subunits of the b_6f complex. The *petB* gene is involved in the production of the cytochrome b_6 of this complex whereas *PetD* is involved in subunit IV located at the thylakoid membrane of the chloroplast (Dumas *et al*., 2018). The cytochrome b_6f oxidized the plastoquinol (PQH_2) in the PSII just like the mitochondrial Q cycle. One electron is given to the b_L hemes of cytochrome b_6 while the other is passed to the Fe-S domain of the Rieske protein. The electrons from the PQH2 shifted to the plastocyanin which carries them to PSI (Tikhonov, 2018). The overall sketch of electron shifting and protons pumping mechanism was well elaborated in Figure 4 (Nelson and Cox, 2017).

Cytochrome b_6 protein has two hemes binding domains represent as b_L and b_H. Two hemes groups b562 and b566 are non-covalently attached to the cytochrome b_6 in the chloroplast thylakoid membrane. The total molecular weight of the cytochrome b_6f complex is 217 kDa in which the cytochrome b_6 subunit has 25 kDa. Approximately 215 amino acid is involved in the production of cytochrome b protein in the chloroplast. The molecular 3D structure of cytochrome b_6f complex in alga *Chlamydomonas reinhardtii* is elaborated in Fig. 5 (MMDB ID# 25730) (https://www.ncbi.nlm.nih.gov/Structure/pdb/1Q90). The following b_6f complex shows the structural similarities to bc_1 complex of mitochondrial respiratory chain. This complex has some additional chlorophyll, beta-carotene, and haem binding sites.

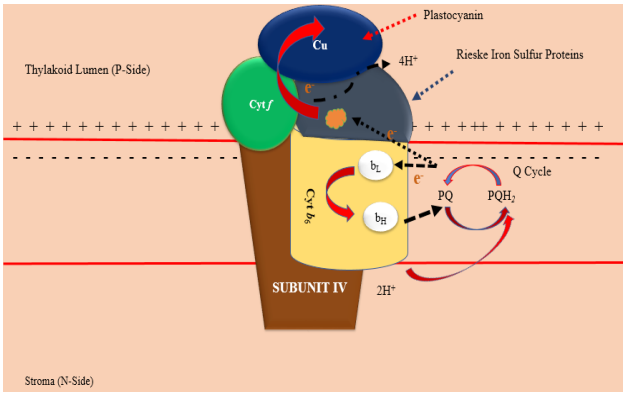

Fig. 4. Electron and proton flow through cytochrome b_6f complex (drawn by the authors).

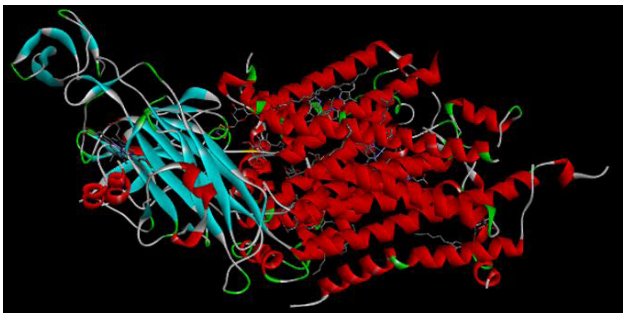

Fig. 5. 3D structure of chloroplast cytochrome b_6f complex in *Chlamydomonas reinhardtii*. Adapted from Protein Data Bank (1Q90) from (https://www.ncbi.nlm.nih.gov/Structure/pdb/1Q90).

Functions of cytochrome b in mitochondrial bc_1 *complex*

The mitochondrial genes have a central role in the production of cytochrome b protein. This protein has a leading role in the synthesis of adenosine triphosphate (ATP). Mitochondrial cytochrome b protein converts the energy from food items into chemical energy. The electron transport chain located at the inner surface of the mitochondrial membrane is mainly involved in the synthesis of ATP (Letts and Sazanov, 2017). The respiratory chain located at the inner mitochondrial surface is composed of four different protein complexes, and free energy is generated by the pumping of protons

across the membrane and by the transferring of electrons between the complexes (Manoj, 2018). This free energy is used for the generation of electrochemical gradients, across the mitochondrial membrane. The mitochondrial ATP synthase located at the inner mitochondrial surface generates ATP from ADP by the addition of phosphate. The proton motive force generated from electrochemical potential is used to run ATP synthase (Klusch *et al*., 2017).

The respiratory chain of the mitochondria possesses complexes I, II, III, and IV at the inner mitochondrial membrane. Complex III also known as bc_1 complex, transfers the electrons from ubiquinone to the cytochrome c reductase in the mitochondrial respiratory chain (Zhu *et al*., 2020). Complex III is made up of membrane-bounded eleven subunits which included cytochrome b, cytochrome c, Rieske protein, two core proteins, and six low molecular weight proteins (Berry *et al*., 2000; Smith *et al*., 2004). The first three respiratory subunits are known as catalytic subunits. The remaining seven non-redox subunits are known as supernumerary subunits because they lack cofactor and are absent in the bacterial electron respiratory chain.

Complex III of the respiratory chain has a unique role in oxidative phosphorylation during which simple sugar is oxidized to produce ATP. The cytochrome b transfers the electrons particles to the complex III in the respiratory chain. Cytochrome b is the only component of the complex III that is encoded by the mitochondrial genome (Lestari *et al*., 2018). The mitochondrial cytochrome b gene region is extensively used in the phylogenetic relationship between different organisms due to its genetic variation. The genetic variation relationship within genera and families also determines through the cytochrome b gene segment. Comparative genomic results showed that mitochondrial DNA is rapidly evolved as compared to nuclear DNA. Therefore, the cytochrome b gene is a valuable marker for species identification and is being used as a marker in ticks, sand flies, mosquitoes, and tsetse flies.

Functions of cytochrome b in chloroplast b_6f *complex*

Cytochrome b_6f complex enzyme also known as plastoquinol-plastocyanin reductase (EC 1.10.99) is present at the thylakoid membrane of the chloroplast in many plants, green algae, and cyanobacteria (Bhaduri *et al*., 2019). This enzyme complex is responsible for the transferring of electrons from plastoquinol (QH_2) to plastocyanin (Pc) within the thylakoid membrane (Kirchhoff *et al*., 2017). The reaction of electrons transferring in the b_6f complex is functionally homologous to the bc_1 complex reaction in the mitochondrial respiratory chain. The overall cytochrome b_6f reaction mechanisms in cyclic and non-cyclic electrons transferring are explained below in the Equations 2 and 3.

$H_2O \rightarrow PSII \rightarrow QH_2 \rightarrow Cyt\ b_6f \rightarrow Pc \rightarrow PSI \rightarrow NADPH$ (For non-cyclic pathway) …(2)

$QH_2 \rightarrow Cyt\ b_6f \rightarrow Pc \rightarrow PSI \rightarrow Q$ (For cyclic pathway) ….(3)

During photosynthesis, the reaction centres of photosystems I and II captures the light energy from the sun with the aid of multi subunits of cytochrome b_6f complex. This complex transfers the electrons from PSII to PSI and pumps the protons across the membrane for the generation of Q-cycle. The reaction in the Q cycle in the chloroplast is like the complex-III of the mitochondrial respiratory chain. This free energy is used for the generation of electrochemical gradients across the thylakoid membrane in the chloroplast (Kanazawa *et al*., 2017). The ATP synthase located at the thylakoid membrane surface generates ATP from ADP by the addition of phosphate.

STRUCTURAL ANALYSIS OF CYTOCHROME B IN TEN MODEL ORGANISMS

Homology modelling and validation of cytochrome b in selected model organisms

The full-length protein sequences of mitochondrial cytochrome b gene, of ten model organisms, were retrieved from NCBI GenBank (https://www.ncbi.nlm.nih.gov) (Sayers *et al*., 2021). Ten model organisms were selected, ranging from archaea to mammals including *Enterobacteriaceae BL21* (bacteria), *Sulfolobus acidocaldarius N8* (archaea), *Saccharomyces cerevisiae* (yeast), *Drosophila melanogaster* (insect), *Danio rerio* (fish), *Gallus gallus* (bird), *Homo sapiens* (mammal; human), *Mus musculus* (mammal; rodent), *Arabidopsis thaliana* (plant), and *Chlamydomonas reinhardtii* (algae) are studied for structural and phylogenetic analysis. The 3D models of these selected cytochrome b were retrieved through online Alpha Fold Protein Structure Database developed by EMBL-EBI and DeepMind (https://alphafold.ebi.ac.uk/)(Fig. 6) (Jumper *et al*., 2021). The PDB file of selected cytochrome b models were opened and 3D models of the selected organisms were displayed into freely available Discovery Studio software (https://discover.3ds.com/discovery-studio-visualizer-download) (Studio, 2008). The model accuracy of cytochrome b in selected model organism were validated through online freely available SAVES server (https://saves.mbi.ucla.edu/). The overall quality factor for non-bonded atomic interaction of all these selected cytochrome b models of were determined through ERRAT server (https://servicesn.mbi.ucla.edu/ERRAT/) (Colovos and Yeates, 1993). The higher quality score of selected cytochrome b models indicating the higher quality of the models (Table I).

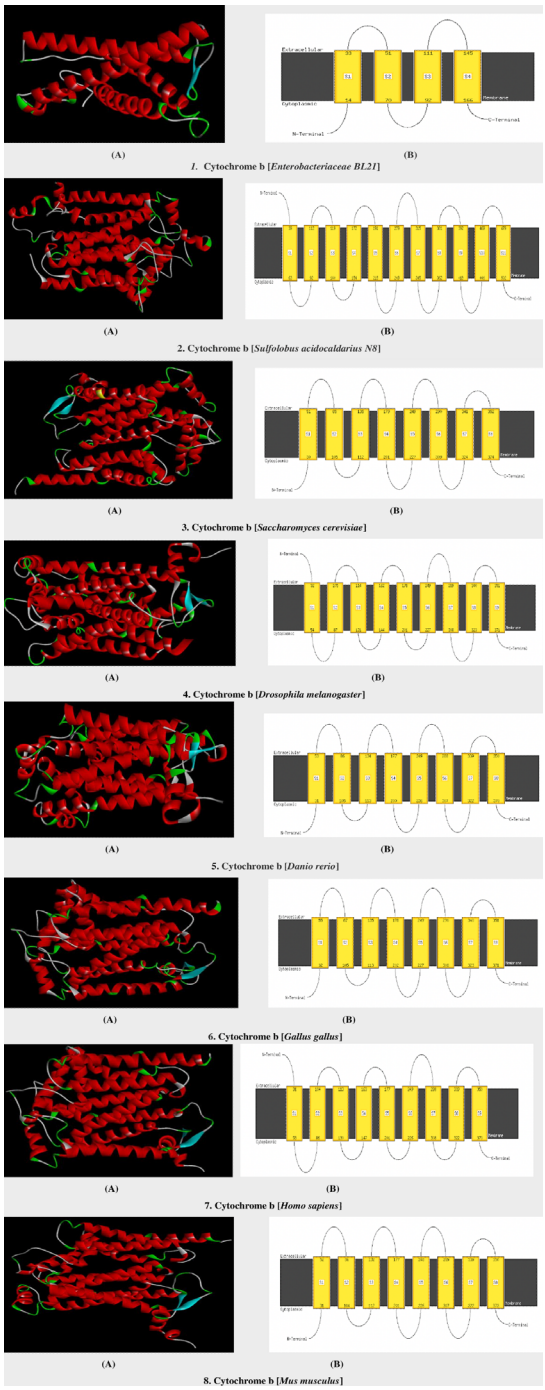

Fig. 6. 3D models and schematic diagram of mitochondrial cytochrome b protein in selected model organisms. A, 3D models and B, the schematic diagram of alpha-helices in selected model organisms.

The position of alpha helices in membrane protein is determined through observing the Phyre2 generated models (Fig. 6). The locations of N- and C- termini were also observed through a schematic diagram generated through this Phyre 2 web tool (Fig. 6).

The stereochemical properties of overall structural geometry of selected cytochrome b models were predicted through PROCHECK server (https://servicesn.mbi.ucla.edu/PROCHECK/) (Laskowski *et al*., 1993). The Ramachandran plot check the stereochemical quality of the protein structure by analysing the geometry between the torsional angles ψ and φ of the residues within the peptide. The stereochemical properties of selected cytochrome b models are good when more than 90% of amino acid residues are in the most favour able region of the Ramachandran plot (Fig. 7). The overall quality factor, amino acid residues percentages in most favoured region, additional allowed region, generally allowed region and disallowed region of Ramachandran plot are shown in Table I. The secondary structures of cytochrome b in all selected model organisms were predicted through online Protein Homology/analogy Recognition Engine (Phyre 2) tool (http://www.sbg.bio.ic.ac.uk/servers/phyre2/html/page.cgi?Id=index) (Kelley *et al*., 2015). The position of alpha helices in membrane protein is determined through observing the Phyre2 generated models (Fig. 6). The locations of N- and C- termini were also observed through a schematic diagram generated through this Phyre 2 web tool (Fig. 6).

Phylogenetic analysis of cytochrome b in selected model organisms

To check the conserved structural residues, the protein sequences of mitochondrial cytochrome b membrane protein are aligned through the online available Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo) (Sievers *et al*., 2011). The position of alpha-helix residues in the aligned protein sequences is highlighted in yellow colour (Fig. 8). Maximum (A to K) transmembrane alpha-helix is observed in archaeal strain *Sulfolobus acidocaldarius N8* and a minimum of (E, F, G, I) transmembrane helices are observed in prokaryotic *Enterobacteriaceae BL21* strain with less homology with other selected model organisms. *Homo sapiens* and *Drosophila melanogaster* have (A to I) transmembrane helices in the mitochondrial cytochrome b transmembrane protein. The remaining six model organisms; yeast, starfish, bird, mammals, plants, and algae contain (A to I) transmembrane alpha-helices except helix D in the mitochondrial cytochrome b protein (Table II). The first transmembrane alpha-helix has glycine, leucine, and threonine residues (G, L, T) highlighted in turquoise colour remained conserved in archaea, unicellular, and multicellular eukaryotes organisms (Table II), whereas the conserved amino acids like tryptophan, leucine, glutamine, and alanine (W, L, Q, A) highlighted in gray shade are only conserved in eukaryotic organisms (Table III).

Table I. Validation of cytochrome b in selected model organisms through ERRAT, and PROCHECK.

Model organisms	Errat	Procheck			
	Quaility factor	Most favoured region	Additional allowed region	Generally allowed region	Disallowed region
Enterobacteriaceae BL21	97.19%	96.20%	3.10%	0.00%	0.60%
Sulfolobus acidocaldarius N8	88.87%	84.70%	12.70%	1.70%	0.90%
Saccharomyces cerevisiae	98.93%	94.70%	4.70%	0.00%	0.60%
Drosophila melanogaster	95.94%	94.50%	5.20%	0.00%	0.30%
Danio rerio	97.04%	94.20%	5.50%	0.00%	0.30%
Gallus gallus	92.45%	85.70%	13.40%	0.60%	0.30%
Homo sapiens	96.50%	94.60%	5.10%	0.00%	0.30%
Mus musculus	96.24%	94.00%	5.70%	0.00%	0.30%
Arabidopsis thaliana	94.50%	92.90%	6.80%	0.00%	0.30%
Chlamydomonas reinhardtii	84.98%	93.80%	5.50%	0.00%	0.60%

Table II. Domain and transmembrane helices of mitochondrial cytochrome b protein in the model organism. Humans, insects, and Archaea have some additional transmembrane alpha-helices as compared to other model organisms. All the model's organisms have N and C- terminal domains in the cytoplasm except humans, insects and archaea have extracellular N-terminal domains.

S. No	Cytochrome B in different model organisms	Classification	No. of helices	Domains	
				N-terminal	C-terminal
1	*Enterobacteriaceae BL21*	Bacteria	4	Cytoplasmic	Cytoplasmic
2	*Sulfolobus acidocaldarius N8*	Archaea	11	Extracellular	Cytoplasmic
3	*Saccharomyces cerevisiae*	Yeast	8	Cytoplasmic	Cytoplasmic
4	*Drosophila melanogaster*	Insect	9	Extracellular	Cytoplasmic
5	*Danio rerio*	Starfish	8	Cytoplasmic	Cytoplasmic
6	*Gallus gallus*	Bird	8	Cytoplasmic	Cytoplasmic
7	*Homo sapiens*	Human	9	Extracellular	Cytoplasmic
8	*Mus musculus*	Mammal	8	Cytoplasmic	Cytoplasmic
9	*Arabidopsis thaliana*	Plant	8	Cytoplasmic	Cytoplasmic
10	*Chlamydomonas reinhardtii*	Algae	8	Cytoplasmic	Cytoplasmic

The *Enterobacteriaceae* species lack helices from one to four (A to D) in multiple sequence alignment results. Structural analysis of the cyt b in model organisms from various kingdoms show that conserved amino acids are mostly located near N- and C-termini residues of the transmembrane alpha-helices. Some residues are also conserved within the helices for e.g., glycine, alanine, and histidine (G, A, H) are conserved within the second transmembrane helices in archaea and eukaryotes while serine, phenylalanine, glycine, and tyrosine (S, F, G, Y) are conserved only in the unicellular and multicellular model organism. The conserved residues in all the helices are highlighted in the multiple sequence alignment results. One interesting feature is that helix D is only observed in humans, archaea, and drosophila which might be the role in coping with extreme environmental conditions. The conserved residues asparagine, leucine, and proline (N, L, P) in helix D are highlighted with dark yellow colour. The conserved residues have a comprehensive role in membrane stability and evolution. The high number of transmembrane helices have a significant role in membrane stabilization. Archaea has two additional helices (J and K) as compared to humans or other eukaryotic organisms which might be leading factors to survive within extreme environmental conditions (Table III; Sr number: (2) The phylogenetic tree of mitochondrial cytochrome b, in all these model organisms, is generated through freely available MEGA-7 software (Fig. 9).

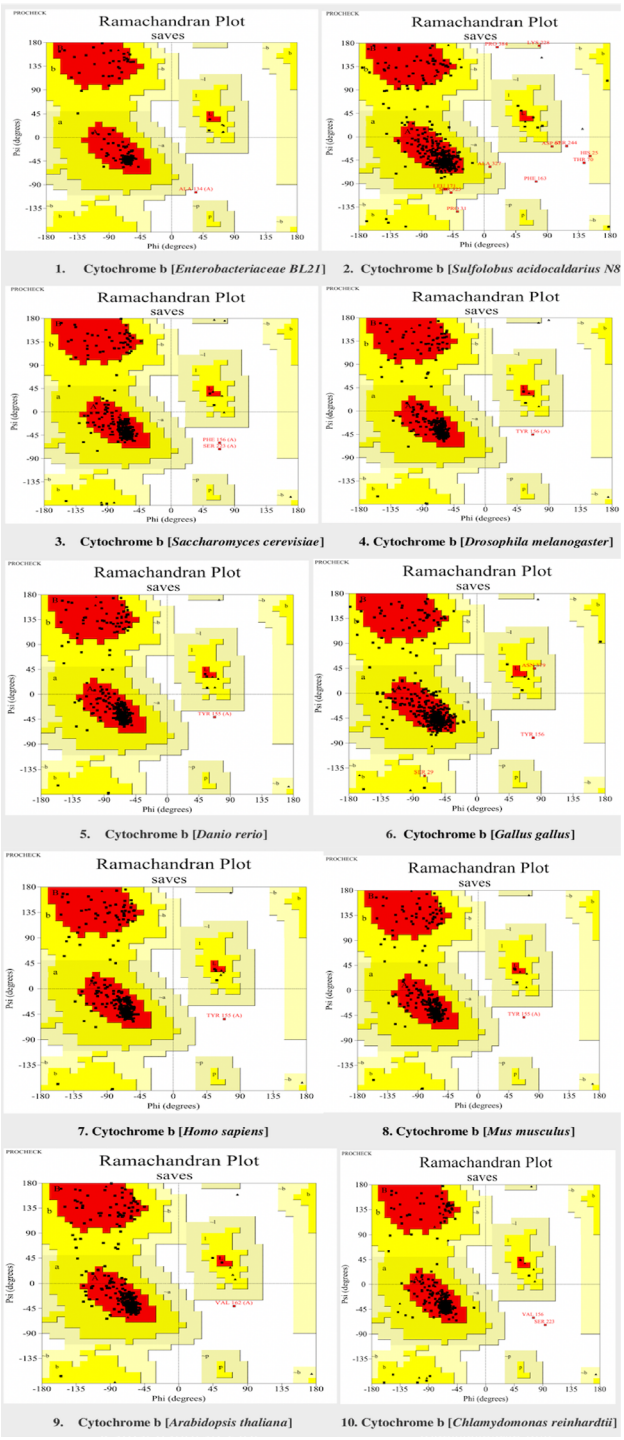

Fig. 7. Verification of cytochrome b models in selected model organisms through Ramachandran plot. Red colour showed the most favoured region (A, B, L). Yellow colour shows the additional allowed region (a, b, l, p). Generally allowed region (~a, ~b, ~l, ~p) is highlighted in light yellow colour. The Ramachandran plots were generated through procheck tool.

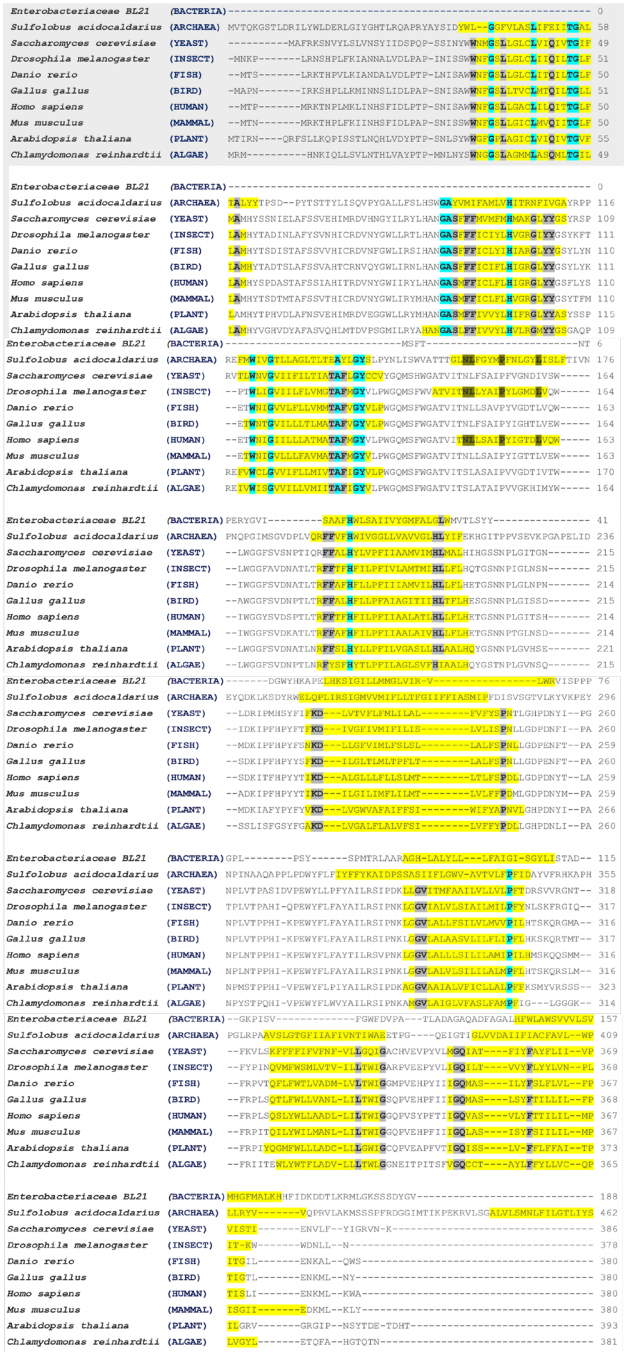

Fig. 8. Multiple sequences alignment of cytochrome b protein in the model organism. Transmembrane alpha-helices in model organisms are highlighted in yellow colour. The conserved residues in archaea and eukaryotic are highlighted with turquoise colour. The eukaryotic conserved residues are highlighted with Gray colour. The dark yellow colour indicates the conserved residues in the high number of transmembrane alpha-helices model organisms. The MSA were performed through the online available Clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo).

Table III. Conserved residues in the transmembrane alpha-helices of mitochondrial cytochrome b protein in the model organism.

Sr. No	Cytochrome b in model organisms	Conserved residues in transmembrane alpha-helices of mitochondrial cytochrome b										
		A	B	C	D	E	F	G	H	I	J	K
1	*Enterobacteriaceae BL21*	Absent	Absent	Absent	Absent	H L	No match	No match	Absent	No match	Absent	Absent
2	*Sulfolobusa acidocaldarius N8*	GLTWLQA	GAHSFGY	WGAYTF	NLP		HFHLKDP	PGV	LG		GQF	Present	Present
3	*Saccharomyces cerevisiae*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFHLKDP	PGV	LG		GQF	Absent	Absent
4	*Drosophila melanogaster*	GLTWLQA	GAHSFGY	WGAYTF	NLP		HFHLKDP	PGV	LG		GQF	Absent	Absent
5	*Danio rerio*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFHLKDP	PGV	LG		GQF	Absent	Absent
6	*Gallus gallus*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFHLKDP	PGV	LG		GQF	Absent	Absent
7	*Homo sapiens*	GLTWLQA	GAHSFGY	WGAYTF	NLP		HFHLKDP	PGV	LG		GQF	Absent	Absent
8	*Mus musculus*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFHLKDP	PGV	LG		GQF	Absent	Absent
9	*Arabidopsis thaliana*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFHLKDP	PGV	LG		GQF	Absent	Absent
10	*Chlamydomonas reinhardtii*	GLTWLQA	GAHSFGY	WGAYTF	ABSENT		HFH	KDP	PGV	LG		GQF	Absent	Absent

The conserved residues in archaea and eukaryotic are highlighted with turquoise colour. The eukaryotic conserved residues are highlighted with gray colour. The dark yellow colour indicates the conserved residues in the high number of transmembrane alpha-helices model organisms.

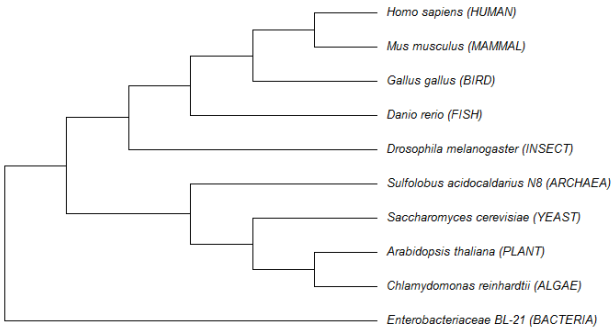

Fig. 9. Phylogenetic analysis by maximum likelihood method of cytochrome b protein of ten selected model organisms. The phylogenetic tree was constructed through MEGAX software. Phylogenetic tree of selected model organism used to study cytochrome b. The tree shows the relative position of some current and alternative model organism to study cytochrome b. The figure predicts that human and mammals are more recent common ancestor than other model organisms.

MITOCHONDRIALCYTOCHROMEBBASED PHYLOGENETIC AND MUTATIONAL ANALYSIS

Polymorphic effects of cytochrome b gene on fertility

The mutation in mitochondrial cytochrome b gene has significant effects on the reproductive system of an organism. Genetic diseases are mostly associated with the substitution and deletions of bases in the DNA (Pal *et al*., 2019). The mitochondrial genome is inherited matrilineally while the nuclear genome is inherited from both mother and father (Wolf *et al*., 2017). Energy is required for the proper functioning of reproductive system of living organisms (Haas *et al*., 2019). The mutation in the cytochrome b gene leads to disturbance in oxidative phosphorylation in mitochondria and consequently other metabolic pathways. In the male reproductive system, the propelling and moving processes of sperm utilizes energy from mitochondria, located at the base of the flagellum (Cardullo and Baltz, 1991). The reduction of energy metabolism leads to defects in male fertility. In *Drosophila melanogaster* the male infertility is observed due to single amino acid substitution from alanine to threonine at position 278 in the protein expressed by cytochrome b gene (Patel *et al*., 2016). This mutation was also observed in the cytochrome b of many other species of vertebrates and invertebrates. The variation in the mitochondrial encoded gene is also responsible for the shorter lifespan of the males in many taxa (Patel *et al*., 2016).

The polymorphism in mitochondrial cytochrome b gene is extensively used for phylogenetic and biodiversity analysis (Jadav *et al*., 2013; Mai *et al*., 2014; Saikia *et al*., 2015). The mutation in the cytochrome b gene affects the male and female reproductive system because it has an equal contribution in transferring and providing the energy in the development of zygote, embryo, and oocytes in females (Ramalho-Santos *et al*., 2009). The mutation in the cytochrome b gene is responsible for the alternation in the post-translation modification like the occurrence of transmembrane helices, phosphorylation site, and leucine-

rich nuclear export signal (Pradhan *et al*., 2018). Recent research has shown that the mutation in the cytochrome b gene induces malfunctioning of the female reproduction of Ghungroo pig. The application of this study has been used as a valuable marker for studying the Ghungroo pig female reproductive system (Pradhan *et al*., 2018).

Cytochrome b gene role in species determination

The mitochondrial cytochrome b gene has a tremendous role in species determination throughout the world. The trading business of endangered species across the world is confirmed through the amplification of the cytochrome b gene. The mitochondrial cytochrome b gene has been extensively sequenced among all the vertebrates. Cytochrome b gene is famous in the scientific world due to its use as a tracer in the phylogenetic relationship (Cutarelli *et al*., 2018). Species identification through mitochondrial cytochrome b gene has a wonderful role in criminal problems, paternity, and maternity testing, illegal trade, lineage, and inbreed counting (Reid, 2018). In many parts of the world species identification is done through the cytochrome b gene (Cutarelli *et al*., 2018). The mitochondrial genome has comparatively less mutational change as compared to the nuclear genome and remains conserved throughout the species. Different mitochondrial genes are involved in species identification like 12S, 16S rRNA, and cytochrome b gene. The cytochrome b gene is extensively used for species identification due to the presence of the stable and variable sequences used by the universal primers. The complete genome size of the mitochondrial cytochrome b gene is approximately 1140 bp (Rahat *et al*., 2020). The amplification of the whole gene is difficult, so the short fragments like 402 bp and 359 bp of the cytochrome b gene is used. These fragments are generated through *Alu*I restriction endonuclease. Different restriction endonucleases have been reported that are used in the cleavages of cytochrome b gene like *Bsa*JI, *Alu*I, *Bst*UI, *Taq*I, and *Bst*NI, *Hin*fI, *RsaI,* and *Nsi*I. The highlighting feature of the cytochrome b gene possesses high polymorphism which makes them key player genes in species identification.

Health conditions associated with cytochrome b gene

The mitochondrial genes are responsible for the production of cytochrome b protein of mitochondrial bc_1complex.The mutations in the complex III encoding genes are responsible for the deficiencies of respiratory enzymes in mitochondria. The deficiencies of this complex III subunit of the mitochondrial respiratory chain lead to neuromuscular and movement disorders in human beings (Bénit *et al*., 2009). The mitochondrial complex III is inherited from the nuclear and matrilineal mode of origin. The complex III respiratory enzyme is produced from one of the mitochondrial cytochrome b gene and nine nuclear genes *BCS1L, UQCRQ, TTC19, UQCRB, UQCRC2, CYC1, LYRM7, UQCC2,* and *UQCC3* (Wanschers *et al*., 2014). Five genes *BCS1L, LYRM7, TTC19, UQCC2,* and *UQCC3* are assembly factors that are involved in the assembly of the complex III subunit of the respiratory chain (Invernizzi *et al*., 2013; Tucker *et al*., 2013). The main highlighting feature of this mutational complex III encoding enzymes is the failure to thrive, bilateral retinal cherry-red spots, and progressive neurodegeneration with Leigh-like brain MRI abnormalities (Mordaunt *et al*., 2015). All the mutations linked to the *TTC19* gene are nonsilent and have a significant contribution to the deficiencies of mitochondrial complex III respiratory enzyme. The severity of the complex III deficiency is linked to the mutation in the mitochondrial cytochrome b gene (Mordaunt *et al*., 2015). The mutated mitochondrial DNA is present with a high percentage in the skeletal muscles which is the clue to identify the myopathies in this individual.

CONCLUSION

In this review article, structural and functional analysis of cytochrome b and their significant contribution in mitochondrial respiratory chain bc_1 and thylakoid membrane of the chloroplast containing b_6f complexes are studied. The present review highlights that the different pet gene is involved in the synthesis of cytochrome b protein. The mitochondrial cytochrome b is encoded by a single *petB* gene whereas in chloroplast *petB and petD* gene is involved in cytochrome b production. The *petD* gene has a fundamental role in the production of subunit IV of the cyt b_6f complex (Δ*petD)* in the thylakoid membrane of the chloroplast. The mitochondrial cytochrome b in bc_1 complexes contains eight transmembrane helices A, B, C, D, E, F, G, and H. Two transmembrane helices B and E in mitochondrial cyt b along with two histidine molecules are crosslinked with two hemes located at the top and bottom of the membrane. Chloroplast b_6f complex contains seven transmembrane helices in which the first four transmembrane helices A, B, C, and D are present in cytochrome b subunit of the b_6f complex whereas subunit IV of the complex contains E, F, and G helices. The reaction of electrons transferring in the b_6f complex is functionally homologous to the bc_1 complex reaction in the mitochondrial respiratory chain. The electrons transferred through these complexes are responsible for the pumping of protons across the membrane-produced ATP through membrane-bounded ATP synthase.

The present review highlights the *in silico* structure

analysis of mitochondrial cytochrome b protein from ten different model organisms is studied starting from bacteria, archaea, yeast, bird, fish, insect, algae, mammal, plant, and humans are predicted through the Alpha Fold 2 and phyre-2 web tool. A detailed structural comparison and sequence alignment of mitochondrial cytochrome b membrane protein is studied through a web-based bioinformatics tool in *Enterobacteriaceae BL21, Sulfolobus acidocaldarius N8, Saccharomyces cerevisiae, Drosophila melanogaster, Danio rerio, Gallus gallus, Homo sapiens, Mus musculus, Arabidopsis thaliana,* and *Chlamydomonas reinhardtii* model organisms. The conserved residues in the transmembrane alpha-helices are predicted through multiple sequence alignment tools. The conserved residues have a comprehensive role in membrane stability and evolution. The high number of transmembrane helices has a significant role in membrane stabilization. Archaea have two additional helices as compared to humans or other eukaryotic organisms which might be leading factors to survive within extreme environmental conditions.

The mitochondrial cytochrome b gene is used as a valuable tool in species identification and phylogenetic analysis throughout the world. The mitochondrial genome has comparatively less mutational change as compared to the nuclear genome and remains conserved throughout the species. The cytochrome b gene is extensively used for species identification due to the presence of the stable and variable sequences used by the universal primers. The mutation in the cytochrome b gene produces a short and abnormal protein which leads to the deficiencies of the complex III protein has some impact reproductive system. The deficiencies of this complex III subunit of the mitochondrial respiratory chain leads to neuromuscular and movement disorders in human beings. *In silico* structure, analysis helps the scientist to better understand the advancement in the evolutionary process. The inheritance of the individuals is strongly linked with the mitochondrial genome. The genome editing through CRISPR/CAS9 technology is the state-of-the-art technology to remove the mutated DNA sequences within the genome. Using this technique, the harmful mutations in mitochondrial cyt b can be edited which opens a new horizon of research to unravel this field.

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