Structural-Functional Characterization of Cytochrome b in bc$_1$ and b$_6$f 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$_6$f 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$_6$f 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$_6$f 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$_6$f 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$^+$) 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$_6$f 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).

$$\text{QH}_2 + 2c^{3+} + 2H^+ \rightarrow Q + 2c^{2+} + 4H_2O \ldots \ldots \ldots (1)$$

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 for its energy currency, and the electron transport chain is a vital pathway for the production of ATP in mitochondria. The electron transport chain is a series of protein complexes that transfer electrons from NADH or FADH$_2$ to oxygen, generating a proton gradient necessary for ATP synthesis. The proton gradient is used by ATP synthase to synthesize ATP from ADP and inorganic phosphate (Pi). ATP synthesis is an essential process for all living cells, as it provides the energy required for various cellular functions, such as muscle contraction, cell division, and active transport. The electron transport chain is composed of five protein complexes: complex I (NADH dehydrogenase), complex II (succinate dehydrogenase), complex III (cytochrome bc$_1$ complex), complex IV (cytochrome c oxidase), and complex V (ATP synthase). The function of each complex is to transfer electrons and protons, generating a proton gradient that drives ATP synthesis. The ATP synthase enzyme uses the energy from the proton gradient to phosphorylate ADP and Pi into ATP. The cytochrome b complex, which is a part of complex III, plays a crucial role in this process by transferring electrons from ubiquinone (QH$_2$) to cytochrome c, which is then reduced to cytochrome c$_{1}$, allowing the transfer of protons across the mitochondrial membrane. This process is essential for the production of ATP, which is used by cells for energy metabolism. The cytochrome b complex is also involved in the production of reactive oxygen species (ROS), which can have both beneficial and detrimental effects on cellular function.
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) that contribute their role in the b,f complex also known as plastoquinone–plastoxyan 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).](image1)

**Structure of cytochrome b in mitochondrial bc₁ complex**

Cytochrome bc₁ 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,f 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 bc₁ 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 bc₁ 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 b₁ and b₂ are present in the first helices bundles. Four histidine residues His84 and His183, His98 and His197 are present in the axial ligands of the b₁ and b₂ hemes and are highly conserved in the cytochrome b gene (Gao et al., 2003). The two species of hemes b₁ and b₂ have different properties and environments within the same protein.

![Fig. 2. Structure of mitochondrial cytochrome b in bc₁ complex (drawn by the authors).](image2)

The mitochondrial cytochrome b in bc₁ 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 b₁ and b₂ subunits, are called first bundles. Q₈ inhibitor site is present in the first bundle near to b₁ hemes site of the cyt b. The remaining three helices F, G, and H
compose the second bundle of the cyt b. Q, site is located near to the b\textsubscript{1} hemes is composed of helices C and F (Ko and Choi, 2016). The lipid bilayer of the mitochondrial membrane is represented as a dotted red line (Fig. 3).

**Fig. 3.** 3D structure of mitochondrial cytochrome b in *Mycobacterium tuberculosis* bc\textsubscript{1} complex (drawn by the authors).

Structure of cytochrome b in chloroplast bc\textsubscript{1} complex

Cytochrome bc\textsubscript{1} 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 bc\textsubscript{1} 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 bc\textsubscript{1} 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 bc\textsubscript{1} complex. The petB gene is involved in the production of the cytochrome b of this complex whereas PetD is involved in subunit IV located at the thylakoid membrane of the chloroplast (Dumas et al., 2018). The cytochrome bc\textsubscript{1} oxidized the plastoquinol (PQH\textsubscript{2}) in the PSII just like the mitochondrial Q cycle. One electron is given to the b\textsubscript{1} hemes of cytochrome b\textsubscript{1} 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).

**Fig. 4.** Electron and proton flow through cytochrome bc\textsubscript{1} complex (drawn by the authors).

Cytochrome bc\textsubscript{1} protein has two hemes binding domains represent as b\textsubscript{1} and b\textsubscript{1}. Two hemes groups b562 and b566 are non-covalently attached to the cytochrome bc\textsubscript{1} in the chloroplast thylakoid membrane. The total molecular weight of the cytochrome bc\textsubscript{1} complex is 217 kDa in which the cytochrome b\textsubscript{1} 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 bc\textsubscript{1} complex in alga *Chlamydomonas reinhardtii* is elaborated in Fig. 5 (MMDB ID# 25730) (https://www.ncbi.nlm.nih.gov/Structure/pdb/1Q90). The following bc\textsubscript{1} complex shows the structural similarities to bc\textsubscript{1} complex of mitochondrial respiratory chain. This complex has some additional chlorophyll, beta-carotene, and haem binding sites.

**Fig. 5.** 3D structure of chloroplast cytochrome bc\textsubscript{1} 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\textsubscript{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 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,f complex

Cytochrome b,f 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 (QH2) to plastocyanin (Pc) within the thylakoid membrane (Kirchhoff et al., 2017). The reaction of electrons transferring in the b,f complex is functionally homologous to the bc, complex reaction in the mitochondrial respiratory chain. The overall cytochrome b,f reaction mechanisms in cyclic and non-cyclic electrons transferring are explained below in the Equations 2 and 3.

\[
\text{H}_2\text{O} \rightarrow \text{PSII} \rightarrow \text{QH}_2 \rightarrow \text{Cyt b} \rightarrow \text{Pc} \rightarrow \text{PSI} \rightarrow \text{NADPH} \quad \text{(For non-cyclic pathway)} \quad \ldots (2)
\]

\[
\text{QH}_2 \rightarrow \text{Cyt b} \rightarrow \text{Pc} \rightarrow \text{PSI} \rightarrow \text{Q} \quad \text{(For cyclic pathway)} \quad \ldots (3)
\]

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,f 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), Saccharobacter acidocaldarius N8 (archaea), Saccharomyces cerevisiae (yeast), Drosophila melanogaster (insect), Danio rerio (fish), Gallus gallus (bird), Homo sapiens (mammal; rodent), Mus musculus (mammal; rodent), Arabopodium thaliana (plant), and Chlamydomononas reinhardtii (algal) 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.ucr.edu/). The overall quality factor for non-bonded atomic interaction of all these selected cytochrome b models of were determined through ERRAT server (https://services.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 1).
Structural Functional Characterization of Cytochrome b in bc₁ and b₆ f Complexes

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 favoured 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 (Phyre2) 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).

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

| Model organisms               | Errat  | PROCHECK  |
|-------------------------------|--------|-----------|
|                               | Quality 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 | N-terminal | Domains |
|-------|------------------------------------------|----------------|----------------|------------|---------|
| 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).
Structural Functional Characterization of Cytochrome b in bc1 and b6 f Complexes

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).
The mitochondrial genome is inherited maternally 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-

| Sr. No | Cytochrome b No in model organisms | Conserved residues in transmembrane alpha-helices of mitochondrial cytochrome b |  |
|-------|-----------------------------------|--------------------------------|---|
| 1     | Enterobacteriaceae BL21 | Absent | Absent | Absent | Absent | H | L | No match | No match | Absent | Absent | Absent |
| 2     | Sulfolobus acidocaldarius N8     | GLTWLQA GASHFGY WGYATF NLPM | HFLHKDP PGV LG | GQF | Present | Present | |
| 3     | Saccharomyces cerevisiae         | GLTWLQA GASHFGY WGYATFF ABSENT | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 4     | Drosophila melanogaster          | GLTWLQA GASHFGY WGYATFF NLPM | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 5     | Danio rerio                     | GLTWLQA GASHFGY WGYATFF NLPM | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 6     | Gallus gallus                   | GLTWLQA GASHFGY WGYATFF ABSENT | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 7     | Homo sapiens                    | GLTWLQA GASHFGY WGYATFF NLPM | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 8     | Mus musculus                    | GLTWLQA GASHFGY WGYATFF ABSENT | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 9     | Arabidopsis thaliana            | GLTWLQA GASHFGY WGYATFF ABSENT | HFLHKDP PGV LG | GQF | Absent | Absent | |
| 10    | Chlamydomonas reinhardtii        | GLTWLQA GASHFGY WGYATFF ABSENT | HFLHKDP 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.

**MITOCHONDRIAL CYTOCHROME B BASED 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 maternally 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-
The complex III respiratory enzyme is produced from one of the mitochondrial cytochrome b gene and nine nuclear genes: *BCS1L*, *UQCRQ*, *TTC19*, *UQCRB*, *UQCRCC2*, *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, and thylakoid membrane of the chloroplast containing b,f 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,f complex (*ΔpetD*) in the thylakoid membrane of the chloroplast. The mitochondrial cytochrome b in bc,f 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 cross-linked with two hemes located at the top and bottom of the membrane. Chloroplast b,f 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,f complex whereas subunit IV of the complex contains E, F, and G helices. The reaction of electrons transferring in the b,f complex is functionally homologous to the bc 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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**Statement of conflict of interest**

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