Bioinformatics Analysis of the Melanocortin 1 Receptor Gene (MC1R) in the Southern Platyfish *Xiphophorus maculatus* (Günther, 1866)

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Abstract: *The Southern platyfish Xiphophorus maculatus is one of the most popular ornamental fish used widely as a premier model system in understanding the genetic basis of color pattern polymorphism. Coloration in X. maculatus is found to be linked with melanin synthesis. Melanocortin genes are involved in the synthesis of melanin pigments. Bioinformatic analysis of the MC1R gene of X. maculatus revealed a 966 bp open reading frame encoding 322 amino acids. The deduced MC1R protein sequence was predicted to possess 7transmembrane G-protein-coupled receptor (7TM GPCR) domain from amino acids 59 to 299 (241 amino acids). The predicted three dimensional structure of the MC1R protein contains seven helices (α1 to α7) from amino acid residues 48-67, 80-102,122-144, 165-187, 197-219, 240-262 and 277-299 respectively within the transmembrane region. In the extracellular region, the presence of amino acid residues from N-terminal position 6-Serine, 8-Leucine, 17-Proline, 20-Glutamic acid, 25-Asparagine and 26-Glutamic acid were predicted to be the binding sites of the receptor. The intracellular region comprising of amino acids residues from 68-79, 145-164, 220-239 and 300-322 interacts with the G protein. The predicted model of the MC1R protein of X. maculatus provides a detailed view of the structure and shows tremendous scope in the field of aquaculture as a target protein/model especially in enhancing fish body coloration.*

Keywords: *Xiphophorus maculatus, MC1R, GPCR, Southern platyfish.*

Introduction

Ornamental fish production is an important component of the aquaculture industry. It contributes positively to rural development in many developing producing countries, and in the major markets for ornamental fish the retail value is many times that of its trade value, with a positive impact throughout the value-chain. The global ornamental fish industry and accessories is worth USD 15 billion and more than 2 billion live ornamental fishes are traded every year. India's contribution is a meagre 0.5% of the world export constituting around Rs.160 lakhs. Thus, an expanding domestic and world market together with the availability of low cost technology for production makes it a promising venture. Presently many countries such as Japan, Malaysia, Singapore, USA and European countries have made the aquarium keeping and ornamental fish culture and trade as a flourishing business [19].

The commercial production of ornamental tropical fish is gaining momentum in many regions of the world. The live-bearing category of ornamental fishes are one of the most popular of all ornamental fishes because they are brightly colored, accepts all kinds of food and breed prolifically to produce free swimming young ones. They are popularly called as "Living Jewels". The live bearing species of the family Poeciliidae such as guppies (*Poecilia reticulata*), mollies (*Poecilia latipinna*, *Poecilia sphenops*), swordtails (*Xiphophorus helleri*) and platies (*Xiphophorus maculatus*) are a popular group being produced in Singapore, Malaysia, Indonesia, Thailand, India and China [2]. India is an emerging country in ornamental fish trade, with its conducive climate and cheap human resource available, is suitable for mass production of exotic ornamental fishes [5]. Among the 288 exotic fish varieties identified in the domestic market of India, Poeciliids such as sailfin molly (*Poecilia latipinna*), swordtails (*Xiphophorus helleri*), guppies (*Poecilia reticulata*), and platies (*Xiphophorus maculatus*) contribute for fifty percent of the market share [17].

Ornamental fish earn high price in the market because of their bright and conspicuous body coloration. This is principally due to the presence of chromatophores found in the dermis, scales, integument, and eyes of fishes [3]. There are two types of Chromatophores namely Biochromes and Schemochromes. Biochromes are true pigments, such as carotenoids (red, yellow, and orange), pterins (white, red, orange, and yellow), purines (white or silver), and melanins (reds, yellows, browns, and blacks). Chromatophores that have the red and orange carotenoids are called erythrophores, while yellow carotenoids are termed xanthophores. Most of the animals do not appear to synthesize carotenoids *de novo* [6] but they obtain carotenoid pigments through different dietary sources such as plant material, algae, and other animals. The melanin pigments are found within the chromatophores called melanophores. Melanin is obtained by the oxidation of amino acids like tyrosine and tryptophan. A fourth type of biochrome called pterinophores, which has pterins is involved in the coloration of eye and other fish tissues. Pterins can be produced in developing pterinophores by vertebrates and other animals [14]. Several fish species have been developed as premier model systems for color expression research, including the guppy *P. reticulata* and the Southern platyfish *X. maculatus*.

The discovery of genes involved in pigmentation polymorphism has increased significantly in the biological evolution of vertebrates [8]. The genes responsible for darkening of coat color have been extensively studied in the laboratory mouse while spontaneous coat-darkening mutations are mentioned in only four genes: The *Agouti* signaling protein (*Agouti),* attractin (*Atrn*), Melanocortin 1 receptor (MC1R) and Mahogunin (*Mgrn*) [1].

The MC1R receptor plays a pivotal role in normal pigmentation. The receptor is primarily located on the surface of melanocytes, which are specialized cells that produces a pigment called melanin. Melanin is the substance that gives skin, hair, and eyes their shading. Melanin is additionally found in the light-sensitive tissue at the back of the eye (the retina), where it assumes a role in normal vision [21]. Melanocytes make two forms of melanin, eumelanin and pheomelanin. The relative quantities of these two pigments determine the coloration. MC1R is a membrane bound-receptor, when it is highly active it signals the melanocyte to produce eumelanin, whereas low activity of MC1R leads to production of pheomelanin or an absence of melanin synthesis [12].

The Southern Platy *X. maculatus* [10] of the genus *Xiphophorus* are small tropical freshwater fish belonging to Mexico, Belize, Guatemala and Honduras. It has been brought into no less than 18 countries and territories including Australia [9]. *X. maculatus* is a small, horizontally

compacted, profound bodied fish with a most extreme body profundity of roughly 50% of the standard length. In the same way as other poeciliids, *X. maculatus* is sexually dimorphic and both males and females may achieve 40 mm and 60 mm separately, however are generally barely smaller (normal roughly 30-40 mm). The Southern platyfish vary extensively in coloration. Female guppies do not express bright color patterns when compared to males. However in platyfish, both the males and females express prominent body coloration. Investigations pertaining to pigment cells in platyfish have set up that various sorts of tinge are communicated, including dark melanins, red, orange, and yellow carotenoids, and yellow, orange, red, and reddish brown pterins [23].

The aim of this research is focused on the MC1R gene from the Southern platyfish *X. maculatus* and to predict the three dimensional structure of the protein by applying various bioinformatics tools. This study would contribute to an understanding of the MC1R protein structure and function involved in body coloration.

Materials and methods

Sequence retrieval

The melanocortin receptor gene in ornamental fishes was searched using GenBank at the NCBI. MC1R of the Southern platyfish *X. maculatus* was selected with GenBank ID: XM_023331925.1 and the sequences were retrieved in FASTA format [16].

Sequence analysis

Bioinformatic analysis of the MC1R gene of the Southern platyfish *X. maculatus* was carried out at the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The Open Reading Frame (ORF) and deduced amino acid sequence was predicted using ORF finder (www. ncbi.nlm.nih.gov/gorf).

Primary structure analysis

Physical and chemical parameters of the melanocortin 1 receptor protein of was calculated by protparam tool and was analyzed for molecular weight, theoretical pI (Isoelectric point), and the grand average of hydropathicitiy (GRAVY) (www.expasy.ch).

Secondary structure prediction

The secondary structure was predicted using SOMPA server [4]. It is used to assess the conformation information of α-helices, β-strands, turns, random and coils within the protein structure. Subcellular localization of the protein was determined with TMHMM Server V. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

Tertiary structure prediction

A BLASTp search of MC1R protein of with default parameter was performed against the Brook Haven Protein Data Bank (PDB) to find the suitable template for comparative or homology modeling. The tertiary structure was predicted using SWISS-MODEL Workspace server [25]. The PDB structure based on sequence identity/similarity were retrieved in PDB format and stored for further analysis. The model was subjected to Ramachandran plot (stereochemistry quality) and was taken into consideration for model validation. RASMOL interface was utilized for viewing the predicted 3D model [18].

Protein model validity

The geometrical and structural consistency of the anticipated model was assessed by different methods. The structural validation was done using RAMPAGE [13], a protein structure verification program. The tertiary structure of MC1R protein of *X. maculatus* has been checked for protein quality by using proQ [24].

Results and discussion

Primary and secondary structure of MC1R protein

The MC1R gene of Southern platyfish has 3373 bp and contained a 966 bp ORF (from 68 to 1036 bp), which encodes 322 amino acids. BLASTp analysis of the MC1R protein revealed to contain a G-protein coupled receptor (GPCR) domain from amino acids 59 to 299 (241 amino acids) (Fig. 1). The MC1R protein was predicted to have a molecular weight of 36.6 kDa. The theoretical pI was found to be 6.84. The Grand average of Hydropathicity (GRAVY) value of protein is 0.785.

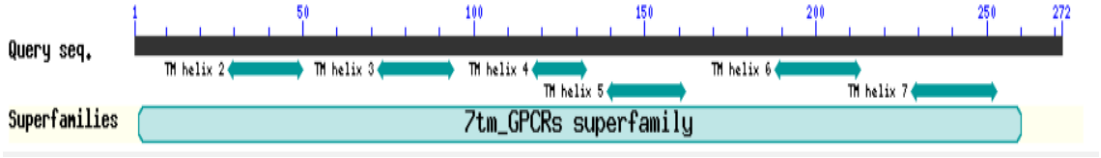

Fig. 1 MC1R protein showing GPCR domain from amino acids 59 to 299

The MC1R, also known as Melanocyte stimulating hormone receptor protein of *X. maculatus* has a molecular weight of 36.6 kDa. The theoretical isoelectric point indicates that the protein is negatively charged. The positive GRAVY value of the protein suggests that it is hydrophobic in nature. This is in accordance to the studies reported earlier in the guppy fish *Poecilia reticulata* [15]. The similarities might be due to the fact that these fishes belong to the same family Poeciliidae. The secondary structure prediction of the entire sequence of MC1R protein using SOPMA (with default parameters) showed the protein having the composition of Helix, Strand, Beta turn and coil represented in Table 1. As evident from this secondary structure prediction, MC1R protein is mostly comprised of alpha helices, extended strands with traces of beta turns.

Table 1. Protein structure composition of MC1R protein of *X. maculatus*

Protein structure unit	Number of amino acids	Percentage of structural unit
α-helix (Hh)	157	48.76
310-helix (Gg)	0	0.00
pI-helix	0	0.00
β-bridge (Bb)	0	0.00
Extended strand (Ee)	66	20.50
β-turn (Tt)	11	3.42
Bend-region (Ss)	0	0
Random-coil (Cc)	88	27.33
Ambiguous states	0.00	0.00
Other states	0.00	0.00

Sub cellular localization of the MC1R protein comprising of 7TM helices and extended strands within the transmembrane region (Fig. 2). The possible N-terminal signal sequence and amino acid sequence position of the MC1R protein of *X. maculatus* are given in Table 2.

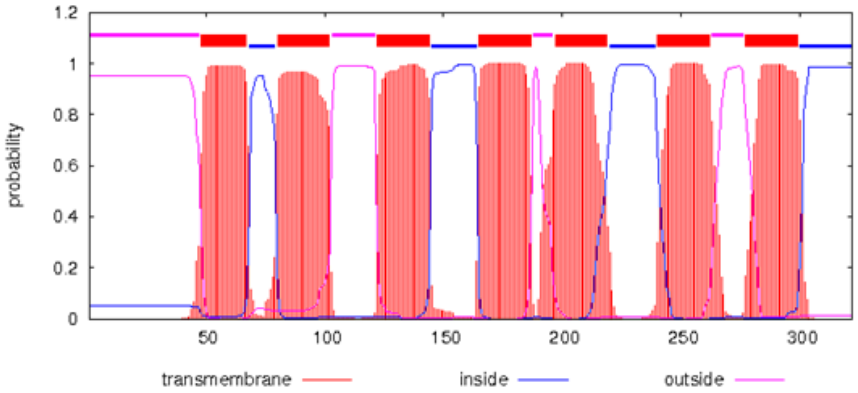

Fig. 2 Transmembrane helices in the MC1R protein of *X. maculatus*

The MC1R protein of *X. maculatus* comprising of 7 helices and extended strands within the transmembrane region as it is evident from the present study. The N-terminal amino acid residues ranging from 1-47, 103-121, 188-196 and 263-276 are extracellular. The amino acid position from residues 48-67, 80-102,122-144, 165-187, 197-219, 240-262 and 277-299 are transmembrane. While, amino acids from 68-79, 145-164, 220-239 and 300-322 are found in the intracellular region. The MC1R is a G-protein coupled receptor that binds to a class of pituitary peptide hormones known as the melanocortins, which incorporate adrenocorticotropic hormone (ACTH) and the diverse types of melanocyte stimulating hormone (MSH). GPCRs constitute a large protein family of receptors that detect molecules outside the cell and activate internal signal transduction pathways and, ultimately, cellular responses. Coupling with G-proteins, they are called seven-transmembrane receptors because they pass through the cell membrane seven times [22].

Table 2. Possible N-terminal signal sequence of MC1R protein of *X. maculatus*

Localization of MC1R protein	**Amino acid sequence position of the MC1R protein**	
Outside	1	47
TM helix	48	67
Inside	68	79
TM helix	80	102
Outside	103	121
TM helix	122	144
Inside	145	164
TM helix	165	187
Outside	188	196
TM helix	197	219
Inside	220	239
TM helix	240	262
Outside	263	276
TM helix	277	299
Inside	300	322

Tertiary structure of MC1R protein

BLASTp analysis of the MC1R protein of *X. maculatus* with the entire amino acid sequence showed good alignment with crystal structure of human A_{2A} adenosine receptor (PDB ID: 3EML A) [11]. Hence, the 3D structure of 3EML A was used as a template to build the model of the MC1R protein using SWISS PDB homology modeling server (ww.spdvi.vitl-it.ch). The predicted 3D structure of *X. maculatus* mostly comprising of α-helix, extended stands of traces of β-turn (Fig. 3).

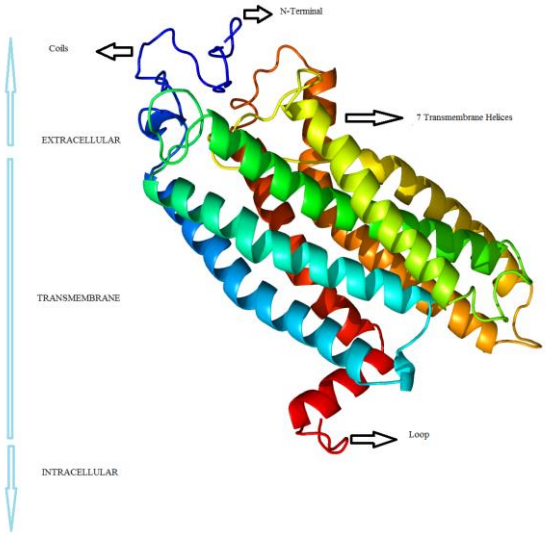

Fig. 3 Tertiary structure of the MC1R protein of *X. maculatus* showing 7TM

The ϕ and Ψ distribution of Ramachandran plot analysis using RAMPAGE revealed that 94.1% and 4.1% amino acid residues are present in the most favored and additionally allowed regions and 1.9% residues in the generously allowed and disallowed regions (Fig. 4). The result shows the predicted LG score 2.453 (> 1.5 fairly good model) and Max Sub score 0.214 (> 0.1 fairly good model) were in acceptable range of a good model.

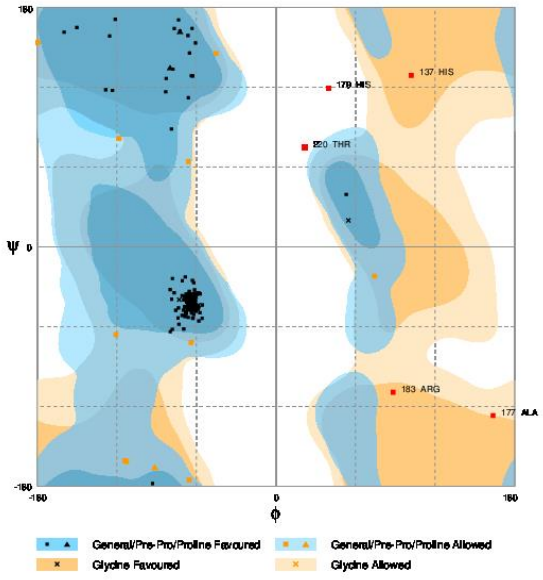

Fig. 4 Ramachandran plot for MC1R protein of *X. maculatus*

The 3D structure of the MC1R protein of *X. maculatus* contains 7 helices (α1 to α7 helices) within the transmembrane region of the cell membrane. The N-terminal of the receptor is present in the extracellular region comprising mostly of coils and loops from amino acid residues 2-6 and 20-39 and traces of helices and extended strands. The presence of amino acid residues in the positions 6-Serine, 8-Leucine, 17-Proline, 20-Glutamic acid, 25-Asparagine and 26-Glutamic acid are predicted to be the binding sites of the receptor. While the C-terminal of the receptor is predicted to be intracellular comprising of loops and few traces of beta turns from amino acid residues 69-70, 175-177 and 292-293, respectively. The 7TM receptor is coupled to the G-protein on the intracellular side of the membrane. When the ligand binds, the GPCR shows a conformational change which is signalled to the G protein causing activation. The predicted tertiary structure of the MC1R protein is in accordance to the crystal structure of human A_{2A} adenosine receptor (PDB ID: 3EML A**)**. The binding site of the Adenosine receptor highlights an integral role of the extracellular loops and helical core in ligand recognition [11]. Similarly, the elucidated structure of MC1R protein in *Poecilia reticulata* extracellular region composed of loops and extended strand, transmembrane regions containing helices and the intracellular region bearing loops and beta turns [15].

MC1R is involved in various physiological processes in vertebrates. Melanocortin are pituitary peptide hormone including adrenocorticotropin and melanocyte-stimulating hormones. In mammals and birds, MC1R is involved in pigmentation and expressed in melanocyte and melanoma Activation of MC1R leads to eumelanin as well as to proliferation and survival of melanocyte in the epidermis [20].

7TM receptors are suitable target for about 50% of all modern medicinal drugs. Their appearance on the cell surface makes them promptly available to hydrophilic medications and their non-uniform appearance gives selectivity in triggering or blocking physiological aspects. Agonists and antagonists of 7TM receptors are employed in the treatment of disease in every organ system. GPCRs are involved in many diseases, and forms suitable target of about 34% of all modern medicinal medicines [7].

Conclusion

In this paper, the 3D structure of the MC1R protein of *X. maculatus* provides a comprehensive view of structure and function with reference to body coloration in ornamental fish. The 3D structure of MC1R protein was authenticated with various bioinformatics tools with an accuracy of 94% (Ramachandran plot analysis) and verified to be a good model (protein quality). Hence, the present investigation creates new vistas in the field of aquaculture to explore further in designing novel peptides and drugs that can be targeted to these membrane proteins to perform a variety of functions in enhancing fish body coloration cell-cell signaling, transportation of ions/solutes, disease diagnosis, treatment and management.

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