

THE STUDY OF COLOUR GENES SEQUENCES IN CHINCHILLA (*CHINCHILLA LANIGERA*) BASED ON HOMOLOGY OF HUMAN AND MICE SEQUENCES

MICHALA POSLUSNA, TOMAS URBAN

Department of Animal Morphology, Physiology and Genetics

Mendel University in Brno

Zemedelska 1, 613 00 Brno

CZECH REPUBLIC

mm.poslusna@seznam.cz

Abstract: In domesticated animals there are many different coat colors and mutations, often connected with pleiotropic effects. The aim of this thesis named The study of color genes sequences in chinchilla based on homology of human and mice sequences was describe by molecular-genetic principles of pigmentation, introduce genes involved in melanogenesis and influencing a melanin function, their structure, mutations and mention other mutations which change the phenotype. Information about alleles *TYR*, *TYRP1*, *TYRP2/DCT*, *agouti*, *AGRP*, gene group *MCR* gene group (*MC1R-MC5R*) and more are focused on human (*Homo sapiens*), mouse (*Mus musculus*) and chinchilla (*Chinchilla lanigera*). In these three species selected sequences of genes *TYR* and *TYRP2/DCT* were compared.

Key Words: chinchilla lanigera, coat color, color mutation, human, melanogenesis, mouse, *TYR*, *TYRP2/DCT*.

INTRODUCTION

Wild animals in nature have a hair color that makes it as inconspicuous as it is possible and blend in with the surroundings for its safety. By domestication there were start to appeal animals with more noticeable hair color, more colorful or patterns and these populations expanded by their attractiveness (Alderton 2011, Ziegler 2014). Genetic variations and mutations which affect final phenotype also changed with this variance. Results of mutations can show attractive hair color or pattern of an animal, but which can may carry some negative consequences because of connection of some mutations with pleiotropic effects, frequently occurring in mammals (obesity, reproductive disorders, deafness, skin sensitivity etc.) (Reismann and Ludwig 2013, Snustad 2009). In human, gene mutations affecting pigmentation mostly cause albinism or red hair (Montoliu et al. 2014). In mice, these gene mutations in addition to albinism cause lighter hair color then wildtype phenotype, color patterns and numerous pleiotropic effects (Ito and Wakamatsu 2011, Beerman et al. 2004).

For location of these mutations of genes there were need to come through the basic molecular-genetic principles of mammal hair pigmentation and mention important genes included in melanogenesis, process of creation of melanins (eumelanin and pheomelanin) and in metabolic pathways which affect these processes. These genes are mainly *ASIP*, *AGRP*, *TYR*, *TYRP1*, *DCT/TYRP2*, *MC1R*, *P/OCA2*, *PMEL17* and *SLC45A2*, which have homologues in mice (in the same order): *agouti*, *Agrp*, *albino*, *brown*, *slaty*, *extension*, *pink-eyed dilute*, *silver* and *underwhite* loci (Slominski et al. 2004, MGI 2016). Mouse is used as a model organism in general for research in different specializations and even in this thesis was used to compare sequences of genes affecting pigmentation in mice, human and chinchilla to make a conclusion, how much are genomes of these three mammals similar. Specifically investigated genes were tyrosinase (*TYR*) and *DCT/TYRP2* (dopachrome tautomerase/tyrosine related protein 2) because of their very high importance in all of the processes.

MATERIAL AND METHODS

In theoretic part of the thesis the molecular and genetic base of pigmentation, melanogenesis and major genes which affect hair and skin color in human and mice are extensively described. Experimental

part of the thesis compares sequences of genes tyrosinase (*TYR*) and dopachrom tautomerase (*DCT/TYRP2*) in three species of mammals: human (*Homo sapiens*), mouse (*Mus musculus*) and chinchilla (*Chinchilla lanigera*). There were found appropriate sequences, which were suitable for comparison, especially sequences of coding DNA (cDNA) in mRNA form in FASTA format using databases NCBI and Ensemble. In case of chinchilla, appropriate parts of whole genome sequence, where is probably located *Tyr* (ChiLan1.0 scaffold00013) and *Dct* (ChiLan1.0 scaffold00003) and predicted mRNA of these genes were found. Further, for chinchilla there was found exact location of all exons of each gene, which is part of a results. For this and for comparison were used databases of NCBI and Ensembl. Sequences of *TYR* and *DCT* in human and mouse were compared together gradually using program BLAST („The Basic Local Alignment Search Tool“), specifically a megablast method (finds highly similar sequences). It is an online tool located on NCBI server (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) for searching similar regions of biologic sequences. It compares nucleotide or protein sequences and calculates statistic indexes (similarity, gaps added by the program) of the accord lines. This tool can be used to assess functional and evolutionary relationships between sequences or it can help to identify gene family's representatives. Program was evolved by Zhang et al. (2000) team. Multiple sequence alignment was made by program Clustal Omega on server EBI (<http://www.ebi.ac.uk/Tools/msa/clustalo/>), where has been made a survey how similar are the investigated sequences. Program was evolved by Li et al. (2009) team. Compared parts of genome were studied in detail, analogy of human, mouse and chinchilla sequences were carefully examined, further mutations and polymorphisms with influence on skin and hair pigmentation in human and hair color in mice were highlighted. NCBI was an excellent tool and source of information interwoven with Ensembl database, which provided a lot of information with great graphic elaboration and simple orientation in the system.

RESULTS AND DISSCUSION

In the experimental part of the thesis we compared sequences of *TYR* and *DCT* gene of the three studied species, first, gradually between them (human – mouse, human – chinchilla, mouse – chinchilla) to find out, how much the sequences similar to each other are. The program evaluated their similarity graphically and statistically. Finally, a multiple sequence alignment for all of the three studied species of mammals together to show the potential similarity was executed.

Gene *TYR* is by NCBI located on 11th chromosome in human, on 7th chromosome in mouse and in chinchilla is unknown, because of no determination of the genomic location. It was found that *TYR* gene contains 5 exons in all the three species of mammals, based on evaluation of the mRNA sequences by the program and there were added the gaps in the regions where were probably missing bases against the comparing sequence by the program. Result is shown in the Table 1. In Table 2 is exact location of *TYR* exons in whole genome chinchilla genome. It was found by comparing whole genome shotgun and predicted mRNA of *TYR* gene. Result of comparison shows that predicted mRNA is placed on the opposite strand against the whole genome shotgun. Finally, was made multiple sequence alignment of sequences of all the three kinds of mammals together, where is not made statistical result, but based on results of each comparing we can say, that the multiple alignment is identical to 83–85%. Further it was found, that match begins with starting codon AUG of the first exon in all the three species of mammals and continues by 4 following successive exons.

Table 1 Evaluation of *TYR* mRNA similarity in three studied species of mammals

Comparison	Similarity (%)	Added gaps
Human – mouse	84	21 (16 – human, 5 – mouse)
Human – chinchilla	85	9 (6 – human, 3 – chinchilla)
Mouse – chinchilla	83	21 (6 – mouse, 15 – chinchilla)

Table 2 Exact location of exons in *TYR* gene in chinchilla

Number of exon	Exon beginning	Exon ending
1	4314001	4313183
2	4302731	4302510
3	4286254	4286096
4	4254596	4254412
5	4244545	4244035

Gene *DCT* is by NCBI located on 13th chromosome in human, on 14th chromosome in mouse and in chinchilla is unknown as *TYR* gene. It was verified, that the gene contains 8 exons in all of three species of mammals. Process was the same like an analysis of *TYR* sequences similarity and result is shown in Table 3. There is the exact location of *DCT* exons in whole chinchilla genome in Table 4. Results show that gene is located on the same strand as whole genome shotgun, which is conversely than result of *TYR* gene. Finally, it was made multiple sequence alignment and, based on results of each comparing, we can assume, that the sequences are identical to 82–86%. Sequence of human and mouse gene are identical in the first exon including the non-coding part. Match of all the three sequences begins by the starting codon of coding part of the first exon and continuous by all 8 following successive exons.

Table 3 Evaluation of *DCT* mRNA similarity in three studied species of mammals

Comparison	Similarity (%)	Added gaps
Human – mouse	83	26 (10 – human, 16 – mouse)
Human – chinchilla	86	6 (3 – human, 3 – chinchilla)
Mouse – chinchilla	82	10 (9 – mouse, 1 – chinchilla)

Table 4 Exact location of exons in *DCT* gene in chinchilla

Number of exon	Exon beginning	Exon ending
1	15359402	15359696
2	15368621	15368919
3	15371477	15371577
4	15372255	15372420
5	15375371	15375550
6	15377101	15377245
7	15390063	15390264
8	15395358	15395556

CONCLUSION

Molecular-genetic basic principles of hair color are identical in all species of mammals. However, genes which affect pigmentation are located in different parts of the genome. Those genes which are the most included in processes of melanogenesis and operation of pigments are analyzed in the thesis. Result of gene analysis by online tools shows gene sequence similarity in the three studied species. Human and mouse genomes and sequences of genes are already well studied, thus localization of genes is known.

But in chinchilla nobody has analyzed the genome and genes for pigmentation yet, so there are available just predicted gene mRNA and probably localized genes. In chinchilla there are not known even mutations of the genes, which are plentiful in human and mouse and affecting final phenotype. Based on high similarity of the sequences of genes we can assume that even in chinchillas occur similar mutations like in human and mouse. After a rigorous detailed study of genes affecting hair color of chinchillas those polymorphisms could be identified in the future and then compared with mutations in mice, what can be the basis for determination of genetic principle of the color mutations in chinchillas.

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