The *agouti* gene in black and brown alpaca.

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Abstract
The *agouti* gene encodes agouti signalling protein (ASIP) which regulates pheomelanin and eumelanin synthesis in mammals. To investigate the role of agouti in coat color variation of alpaca, we characterised the *agouti* gene on 27 black and 12 brown alpaca. The exon-4 hosts three loss-of-function recessive mutations: g.3836C>T, g.3896G>A and g.3866_3923del57, involved in eumelanin synthesis. The deletion at the position p.C109-Rdel19 eliminates the two beta sheets and the R-F-F- motif from the agouti functional domain, which are essential against alfa-MSH. Therefore, the deleted allele appears to lose function. The other ANPs observed at the amino acid position 98 and 118 change the conserved R to C and the R-F-F- motif into H-F-F-. The R-F-F- motif is important for functioning at MCRs; the disruption of this motif may result in a non functional agouti protein since the alteration of residues in and around R-F-F- causes a decrease in agouti protein inhibition of alfa-MSH binding to MCRs during signal transduction. The three mutations are randomly distributed among the black alpaca. In our sample, we observed two genotypes : g.3836C>T/g.3896G>A (10 animals) and g.3836C>T/g.3866_3923del57 (17 animals). Among the brown alpaca, 2 are homozygous for the wild allele, 12 are heterozygous for g.3896G>A mutation, carriers for black phenotype.

Resumen : Gen Agouti en alpacas negra y marrón
El gen *agouti* codifica la proteina señalarizadora (ASIP) que regula la sintesis de la feomelanina y la eumelanina en mamíferos. Para investigar el rol de *agouti* en la variación del color de capa en alpaca, caracterizamos el gen agouti en 27 alpacas negras y 12 alpacas marrones. El exón 4 presenta tres mutaciones recesivas que provocan pérdida de función : g.3836C>T, g.3896G>A y g.3866_3923del57, involucradas en la sintesis de eumelanina. La deleción en la posición C109-Rdel19 elimina dos β-láminas y el motivo estructural R-F-F- del dominio funcional del agouti, que son esenciales frente al alfa-MSH. Por lo tanto el alelo que porta la delecion manifesta la pérdida de funcion. Los ANPs observados en la posicion de los aminoacidos 98 y 118 cambian la region conservada R a C y los motivos estructurales R-F-F en H-F-F-. La estructura supersecundaria R-F-F- es importante para el funcionamiento del MCRs; la disrupción de ese motivo estructural puede resultar en una proteina agouti no funcional ya que la alteración de residuos alrededor y en R-F-F- causa una disminución en la inhibición de la proteína agutí en el sitio de union alfa-MS a MCR durante la señal de transducción. Las tres mutaciones en alpacas negras, se distribuyen al azar . En nuestra muestra, observamos dos genotipos: g.3836C>T/g.3896G>A (10 individuos) y g.3836C>T/g.3866_3923del57 (17 individuos). Entre las alpacas marrones, 2 son homocigotas para el alelo silvestre, 12 son heterocigotas para la mutacion g.3896G> A, portadores del fenotipo negro.

Keywords: agouti, SNPs, duplication.

Introduction

There are two major types of melanin pigment produced by mammalian pigmented cells: black/brown eumelanin or red/yellow pheomelanin. Both require the enzymatic oxidation of tyrosine to form dopaquinone. During pheomelanin synthesis, dopaquinone is produced at relatively low levels and becomes incorporated into sulfhydryl derivates. In contrast, eumelanin synthesis is associated with a high rate of dopaquinone production and subsequent enzymatic oxidation into indole derivates. Studies based on coat color mutations in mice have identified several genes that regulate whether melanocytes produce pheomelanin or eumelanin (Montoliu et al., 2011).
A focal point for pigment type switching is the Melanocortin 1 receptor (Mc1r), formerly known as Extention, which encodes a seven-transmembrane-domain receptor expressed by hair follicle melanocytes; gain-of-function Mc1r mutations cause exclusive production of eumelanin, whereas loss-of-function mutations cause exclusive production of pheomelanin. The Agouti gene encodes the ligand for the Mc1r and is a paracrine signalling molecule secreted by mesenchymal cells in dermal papillae. Agouti protein inhibits Mc1r function such that gain-of-function Agouti mutations cause exclusive production of pheomelanin, whereas loss-of-function mutations cause exclusive production of eumelanin. Other additional molecules are required for Agouti inhibition of Mc1r function, like Attractin (Atrn), which acts as an accessory receptor for Agouti protein, or Mahoganoid (Mgrn1), an intracellular protein with E3 ubiquitin ligase activity (Montoliou et al., 2011).
A preliminary characterization of full length *agouti* transcripts (i.e. including 5' and 3'UTRs beside the coding region) from skin biopsies of multi coloured population of Peruvian alpaca (Bathrachalam et al., 2010; Bathrachalam et al., 2011) and on exclusively the coding sequences of the same gene from genomic DNA isolated from blood samples of Australian alpaca population have been recently realised (Feely et al., 2011). Genomic locus structure, transcripts and causative mutations of the gene are finally defined by Bathrachalam et al., 2013.
In this study, we report the identification of mutations that probably affects eumelanin and pheomelanin synthesis from the black and brown native Peruvian alpaca.
Brown and black alpacas were sampled from ILPA-Puno, Quimsachata Experimental Station, Instituto Nacional de Innovacion Agraria (INIA), Peru which is located 4300m above to the sea level.

Characterization of the agouti full length transcript and genomic locus

The combined transcript sequence obtained from total RNA purified from lateral skin of brown alpaca is 822 bp long (excluding poly A tail). It is composed of 402 bp open reading frame (ORF), a 196 bp 5'UTR and a 224 bp 3'UTR (Fig.1). It encodes a putative 133 amino acid protein.
The amplification of genomic DNA using AF1/AR1 primers displayed a 3945 bp fragment (GenBank accession no: HQ645014), which contains three coding exons (2, 3 and 4), in addition to intronic sequences. The coding exons 2, 3 and 4 are separated by 1262 and 2281 bp intronic sequence, respectively. Each exon is flanked by consensus splice donor and acceptor sites, except for the exon 4, which has only a splice acceptor site (Fig.2).

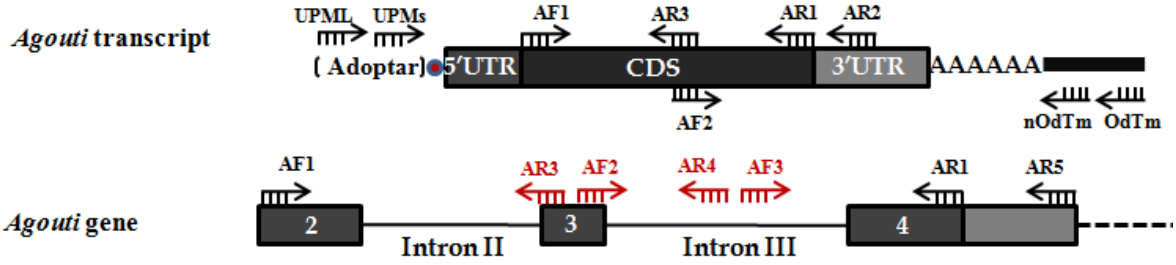

Figure.1. *Schematic view of agouti transcript and gene amplification.* ***Agouti transcript:*** *A schematic picture represents the strategy followed in the full length transcript amplification (CDS, 3'UTR followed by 5'UTR). The primers (forward & reverse) and its positions mentioned above to the cDNA structure are used in the CDS and 5'UTR amplification. Primers and it location mentioned below to the cDNA structure are used in the 3'UTR amplification.* ***Agouti gene:*** *A schematic picture of the agouti gene amplification. The primers mentioned in black colour are used in the amplification and the primers mentioned in the red colour are used in the sequencing of the amplified gene.*

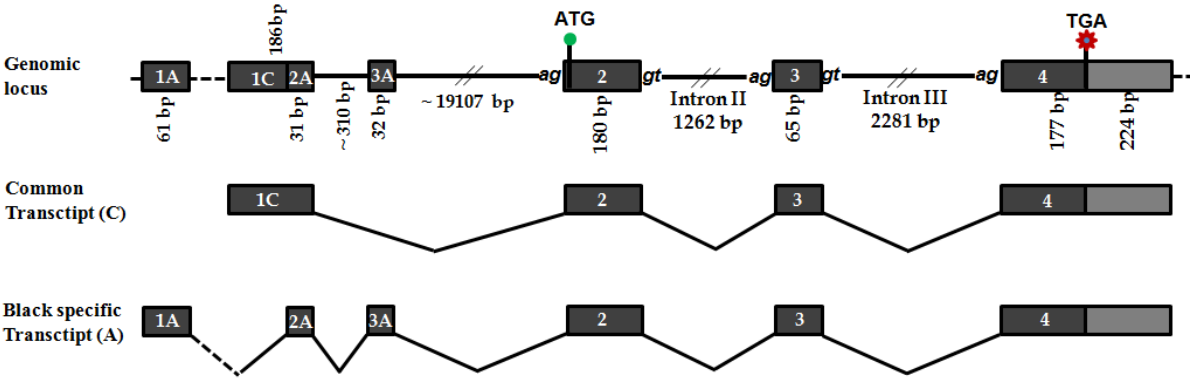

Figure.2. *The alpaca agouti genomic locus and its transcripts. Numbers above and below the gene structure are length of exons and introns. Consensus acceptor and donors splice sites are identicated. Boxes show coding exons (2, 3 and 4) and 5'UTR exons (1A, 2A, 3A and 1C). Transcript C and A are represented under the genomic locus. The position of coding exons (2, 3&4), introns (2&3) and 3'UTR are deduced from our experiment. The positions of non coding exons (2A, 3A and 1C) are deduced based on the ensemble 2X alpaca genome. The position of the non coding exon 1A is not known (---).*

Polymorphisms screening

Initially, we characterized the whole coding sequence of *agouti* cDNA in 35 multi colored (different shades from eumelanin to pheomelanin) Peruvian alpacas. In which, we observed 10 single point mutations, among those three were found to be silent mutations, four were missense mutations and three were observed in the 3'UTR (Bathrachalam et al., 2010). The mutations observed in cDNA correlates only with eumelanic and pheomelanic traits in general and not with the individual coat color. Hence, we started to analyse the *agouti* from genomic DNA of brown and black. The sequence comparison (from ATG to TGA) revealed

in total 19 single nucleotide polymorphisms (SNPs) and one in-frame 57 bp deletion at the position g.3866-3923 in the exon-4 (cystine rich domain) (Fig. 3). In which a synonymous SNP g.102G>A was observed in the exon-2, 6 SNPs (g.458T>C, g.589T>A, g.594G>A, g.681T>C, g.1000G>A, g.1158C>T and g.1179T>C) were observed in the intron-2, 5 SNPs (g.1571C>A, g.2375A>G, g.3208C>A, g.3278T>C and g.3292T>G) were observed in the intron-3 and 3 SNPs were observed in the exon-4 among those g.3835C>A was synonymous and g.3836C>T and g.3896G>A were observed to be non synonymous. Apart from these, mutations were also identified in the 3'UTR. Among those one was a transversion and two were transition mutations located at 10 (g.3955C>A), 38 (g.3983A>G) and 77 bp (g.4022T>C) downstream to the stop codon. The SNPs (g.3836C>T and g.3896G>A) and an in-frame 57 bp deletion (g.3866-3923del57) in exon-4 are predicted to independently cause functional changes to agouti protein. The g.3866-3923del57 would result in a short 114 amino acid containing agouti protein, which lacks 19 amino acids p.C109_R127del19 (CDPCAFCQCRFFRSVCSCR) from the cysteine (C) rich domain, which is critical in agouti function. The g.3836C>T SNP would predict a change of arginine (R) to C, which would disrupt the highly conserved region of the protein. And the other SNP g.3896G>A changes the R to histidine (H) in the cystine-rich domain, which disrupt the highly conserved Arg-Phe-Phe (R-F-F) motif in the protein, respectively.

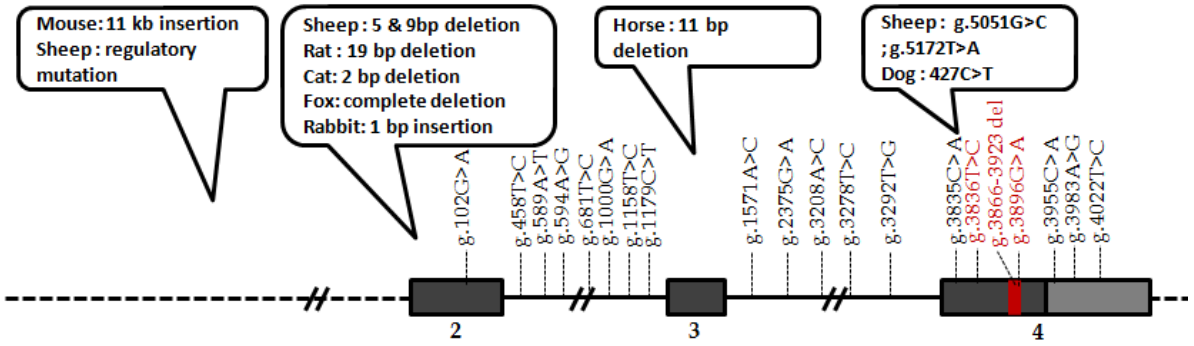

Fig.3. *The alpaca agouti gene and polymorphism. Polymorphisms identified in the different coloured alpacas are mentioned on the genomic organization of the Agouti deduced from our study. Nonagouti mutations identified in other species are reported above to the corresponding position related to the alpaca gene structure.*

Functional analysis and molecular modelling

In silico functional analysis by PANTHER, cSNP of the p.R98C and p.R118H supports the putative functional role of these mutations (p.R98C, subSPEC = −3.67256; $P_{deleterious}$ = 0.66208 and p.R118H, subSPEC = −6.36458; $P_{deleterious}$ = 0.96658) and the fact was further evidenced by SNAP tool (p.R98C, non-neutral; RI, 3; EA, 78% and p.R118H, non-neutral; RI, 6; EA, 93%). These prediction suggests that the mutation at the position g.3896G>A may produce a non functional agouti and the g.3836C>T variant may produce agouti protein with minimal/partial activity. The protein sequences of alpaca agouti with mutation at the position C98 and agouti sequence (pdb structure 2kza) were submitted to CPHmodels 3.0 Server (Lund et al., 2002; Nielsen et al., 2010). The phi, psi angles at this mutated position were checked using the software Ramachandran Plot 2.0 (http://dicsoft1.physics.iisc.ernet.in/rp/). There is not much difference in these torsion angles between the mutated and normal sequence and could possibly have less impact on the structure and function of the protein.

The quality of the model was further checked with WHATIF (Vriend, 1990) which reaffirmed the quality of the model obtained from CPH models server (Nielsen et al., 2010). The original amino acid R97 in 2kza shows hydrogen bonding with C125 but the mutated residue at this position from the model shows simulated hydrogen bonding with two amino acids such as C125 and R126. Hence, the p.R98C variant seems to have minimal/partial effect on the functional property.

Association analysis of g.3836C>T, g.3896G>A and g.3866_3923del57 with phenotypes

A coat color panel comprising 39 DNA samples from brown (12) and black (27) Peruvian alpacas were genotyped for g.3836C>T and g.3896G>A SNPs and g.3866_3923del57. Ten out of twelve brown animals were heterozygous to g.3836C>T, homozygous for the SNP g.3896G, two animals were homozygous to g.3836C and g.3866_3923del57 was not observed. In black animals we found 2 genetic background (i) black heterozygous to g.3866_3923del57 *agouti* allele and (ii) black homozygous to undeleted *agouti* allele. We further investigated this variation in the black individuals to get more insights, from that we identified the *agouti* allele without in-frame deletion present in the g.3866_3923del57 animals has (g.3836C and g.3896A) disrupted R-F-F motif. This condition supported that other allele observed in the g.3866_3923del57 ($a^{\Delta 57bp}$) black could be non functional. In other black which is homozygous to the undeleted *agouti* allele is heterozygous to both the SNPs (g.3836C>T and g.3896G>A). Further investigation with cloning experiment unveiled that one of the allele with g.3896G>A (a^{H}) seems to be non functional due to the R-F-F motif disruption. The other allele seems to have partial/minimal functionality; this situation could explain the two genetic backgrounds found in the black Peruvian alpaca population.

Discussion

In this paper, we report the mutations that affect coat color in native Peruvian alpaca population.

In agouti protein, cysteine-rich domain alone is sufficient for high-affinity binding and activity at the respective melanocortin receptors (MCRs) (McNulty et al., 2001; McNulty et al., 2005; Jackson et al., 2006; Patel et al., 2010). The amino acid deletion/insertion/substitution in this region possibly results in significant functional alteration. In our population the identified polymorphisms/deletion at C-rich domain may have functional importance. The deletion at the position p.C109_Rdel19 eliminates the two β-sheets and the R-F-F motif from the agouti functional domain, which are essential to play the antagonist role against α-MSH (McNulty et al., 2005). Therefore, the deleted allele appears to result in loss of function. The other SNPs observed at the amino acid position 98 & 118 changes the conserved R in to C and R-F-F motif into H-F-F. The R-F-F motif is important for function at MCRs; the disruption in this motif may results in non functional agouti protein. Since, the alteration of residues in and around R-F-F causes decrease in agouti protein inhibition of α-MSH binding to MCRs (Kiefer et al 1998) during signal transduction.

The extensive characterization of loss-of function *agouti* mutations in mice realised by Miltenberger et al. (2002) found that one allele containing an‘‘unpaired cysteine’’ (C115S), retained a very small amount of biological activity as manifested by the presence of small amounts of pheomelanin synthesis in the perimammary and perineal areas. In our previous

preliminary report, the molecular modelling of alpaca agouti domain shows no appreciable change in the protein structure with p.R98C substitution (Bathrachalam et al., 2009). Further analysis with torsion angles did not show much difference between the mutated and normal sequence, hence this mutation could possibly have less impact on the structure and function of the protein. The above mentioned fact for p.R118H is further confirmed with the SNAP prediction (non-neutral) reliability index (6) and expected accuracy (93%); cSNP subSPEC (-6.36458) and $P_{deleterious}$ (0.96658). But for the other SNP R98C seems to maintain partial/minimal agouti function. Since the SNAP prediction (non-neutral) reliability index (3) and expected accuracy (78%); cSNP subSPEC (-3.67256) and $P_{deleterious}$ (0.66208).

In the brown phenotypes (A/a^{ht}) one allele (A) seems to be functional and the other (a^{ht}) seems to retain partial/minimal function, since they are heterozygous to p.R98C. This condition portray that the allelic partial dominance/haploinsufficiency could be the possible explanation for brown phenotype. On the other hand two genetic backgrounds are observed in the black phenotypes i.e. *non-agouti* black (high eumelanin) with deletion that includes the R-F-F disruption and the other observed black (low eumelanin) possibly due to partial dominance/ haploinsufficiency.

We did not observe any homozygous animals for the *non-agouti* alleles in Peruvian alpaca population studied. Our cloning experiment clears that the mutations (p.R98C & p.R118H) in black phenotypes are alternatively arranged. The a^{hT} allele in the black phenotypes possibly having the partial dominance/ haploinsufficiency effect on the eumelanin synthesis due to its minimal function. In the same way, the heterozygous condition of the mutation (p.R98C) observed in brown animals could have the same effect. Since, reddish brown animals are observed to have the wild type (A/A) alleles and the partial dominant mutation (a^{hT}) on reddish brown may lead to light reddish brown that can be often recognised or misidentified by the breeders/farmers as brown.

In conclusion, the characterization of the *agouti* gene provides a clear picture about eumelanic and pheomelanic genetic backgrounds and probable role in coat color phenotypic variation of alpaca. The identification of *non-agouti* mutation in alpaca represents an important step in the development of marker assisted breeding programme for coat colors.

Acknowledgement

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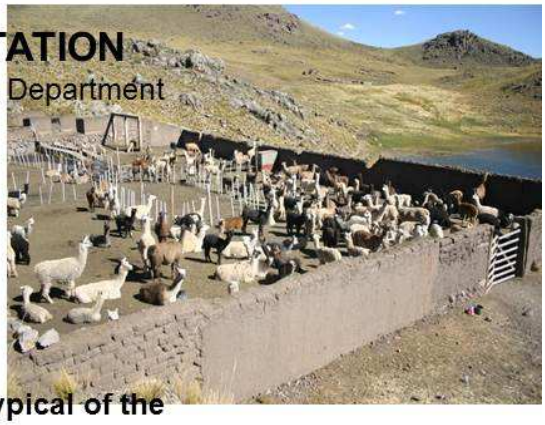
The *agouti* gene in black and brown alpaca

Nantes
August 29, 2013

QUIMSACHATA STATION

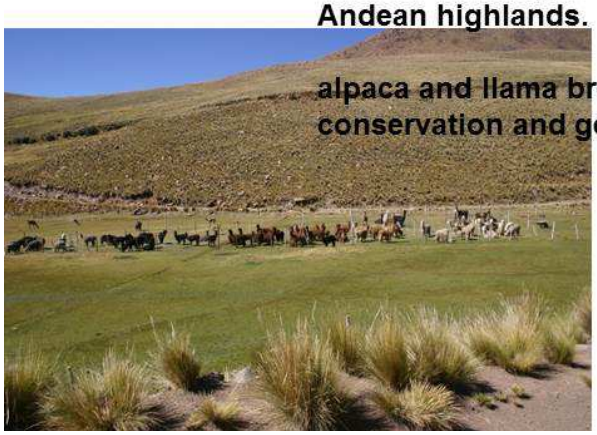
Santa Lucia District, Puno Department



6,282 hectares

4400 m above sea level

Grassland ecosystem, typical of the Andean highlands.



alpaca and llama breeding, conservation and genetic improvement.

145 offspring born from

4 white sires x 36 white dams,

4 white sires x 39 pigmented dams,

9 pigmented sires x 70 pigmented dams
4 black sires x 25 black dams,
2 black sires x 20 brown dams,
3 brown sires x 25 brown dams.

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- **BATHRACHALAM C., RENIERI C., LA MANNA V., LA TERZA A. 2013.** ***The alpaca agouti gene: genomic locus, transcripts and causative mutations of eumelanic and pheomelanic coat color.*** **Gene, doi:10.1016/j.2013.03.060**.

MELANOGENESIS

Melanin pigments can be classified into two major groups:

brown to black insoluble EUMELANINS

alkali-soluble yellow to reddish-brown PHEOMELANINS.

Both pigments derive from the common precursor DOPAQUINONE

formed via the oxidation of L-tyrosine by tyrosinase.

EUMELANINS are black or brown nitrogenous pigments, insoluble in all solvents, which arise by oxidative polymerization of 5,6-dihidroxyindoles derived biogenetically from tyrosine via dopaquinone

PHEOMELANINS are alkali-soluble, yellow to reddish brown pigments, containing sulfur in addiction to nitrogen and arising by oxidative polymerization of cysteinyldopa via 1,4-benzothiazine intermediates

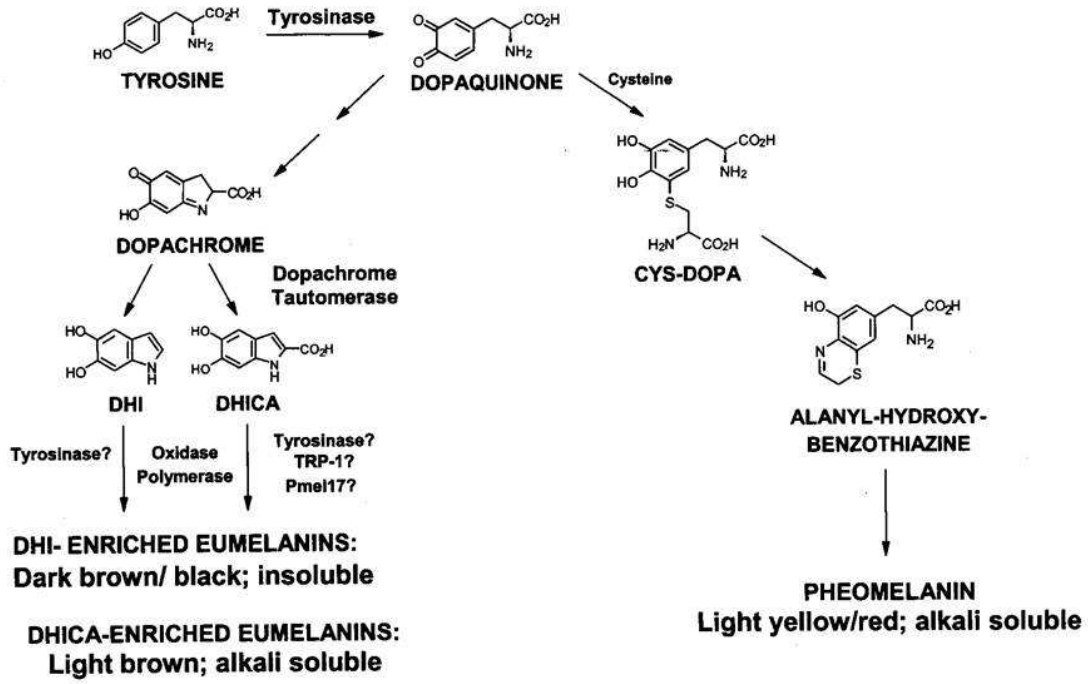
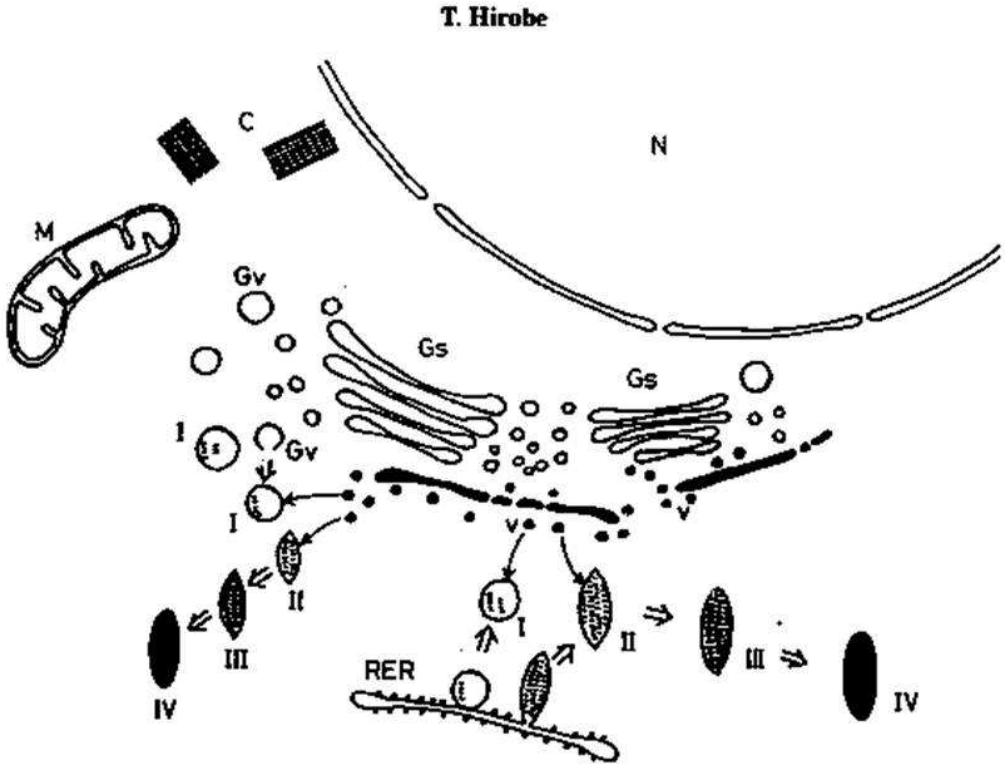


Fig. 1. The melanin synthetic pathway.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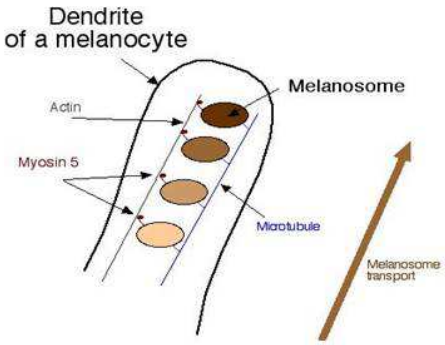
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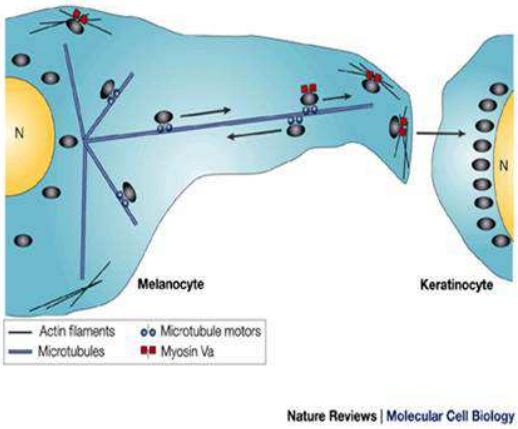
Myosin 5
Microtubule
Melanosome
transport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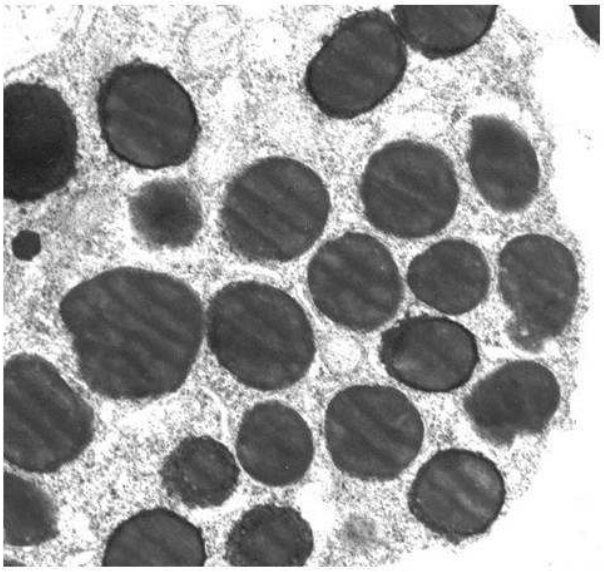
Nature Reviews | Molecular Cell Biology

EUMELANOSOMES (BLACK ALPACA)

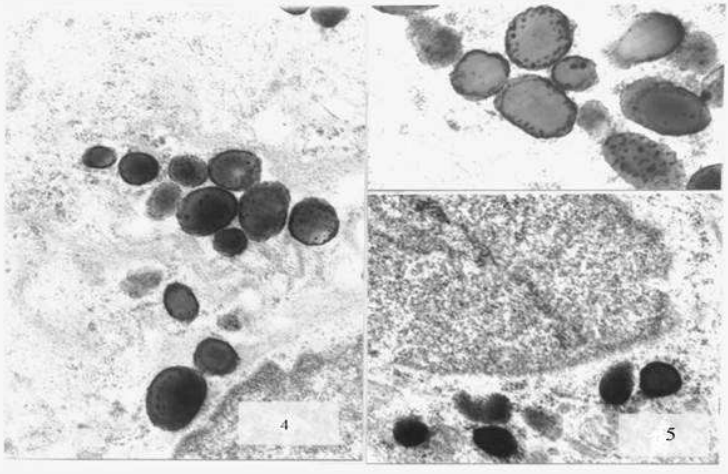


OVAL IN SHAPE

SURRONDED BY MEMBRANE

CHARACTERISTIC STRIATION

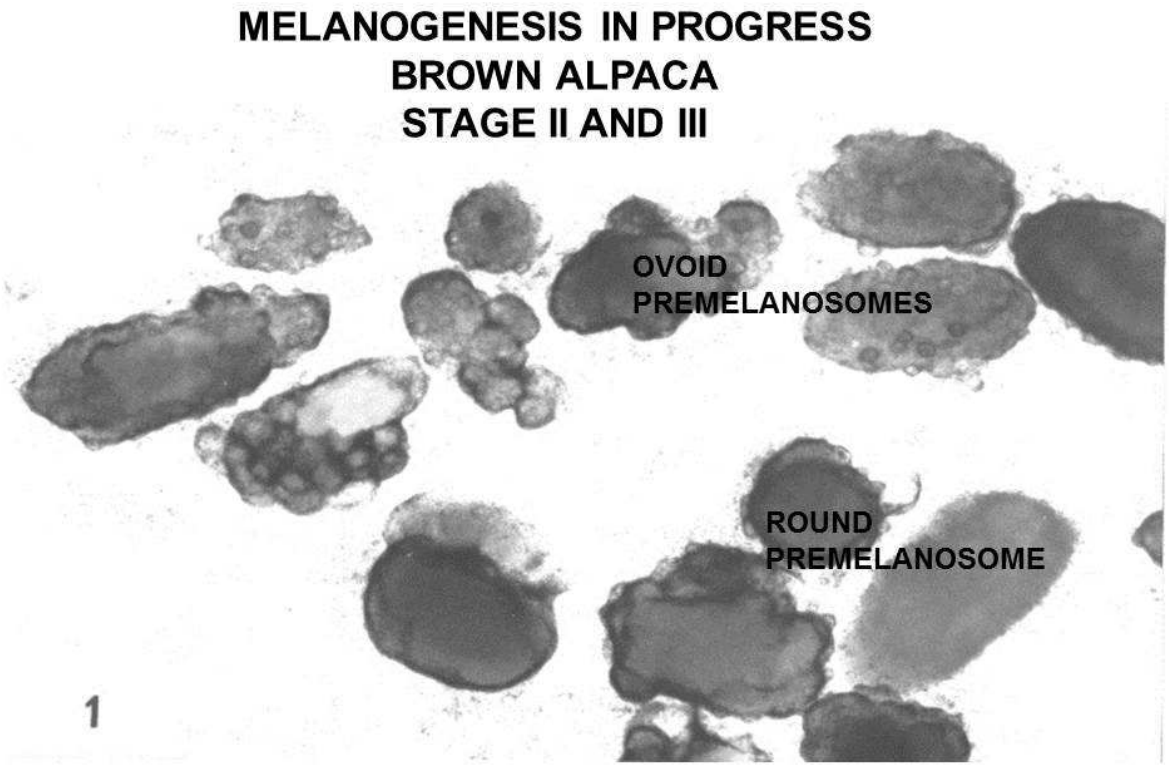
PHEOMELANOSOMES (BROWN ALPACA)

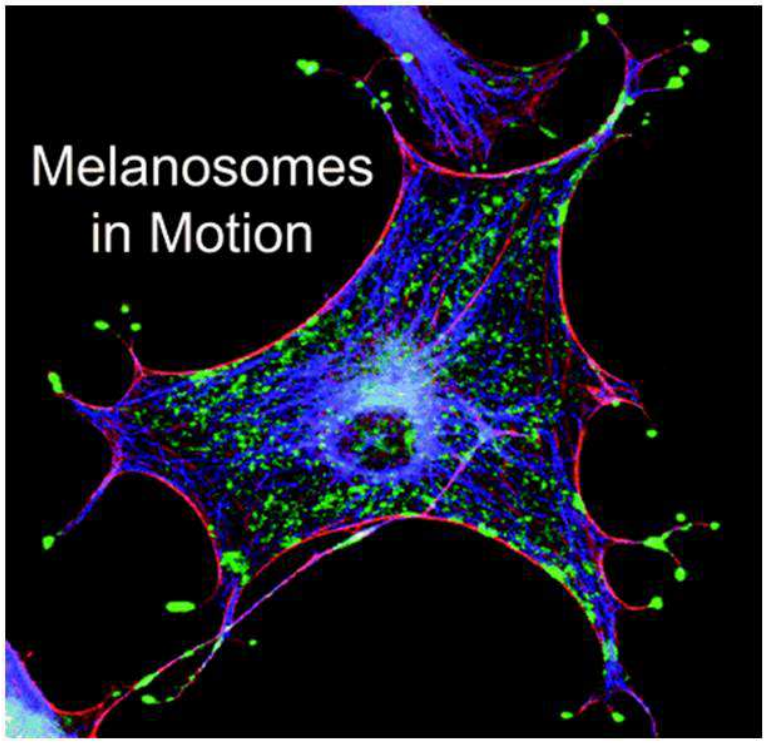


ROUD SHAPE

PALE MATRIX

ELECTRON DENSE DOTS LOCATED NEAR THE LIMITING MEMBRANE


MELANOGENESIS IN PROGRESS
BROWN ALPACA
STAGE II AND III
OVOID
PREMELANOSOMES
ROUND
PREMELANOSOME
1


Melanosomes
in Motion

THE SWITCHING FROM PHEOMELANOGENESIS TO EUMELANOGENESIS IS CHEMICALLY CONTROLLED BY THE CYSTEINE CONCENTRATION.

Dopaquinone plays a pivotal roles on chemically controlling melanogenesis :

sulfyydryl compounds are absent,
- it undergoes intramolecular cyclization to form CYCLODOPA,
- which is rapidly oxidized by redox reaction with dopaquinone to give DOPACHROME (and DOPA).
- Dopachrome then gradually rearranges to give mostly 5 ,6-DIHYDROXYINDOLE (DHI) and a trace of 5,6-DHIHYDROXYINDOLE-2-CARBOXYLIC ACID (DHICA).
- Oxidation of these dihydroxyindoles leads to the production of EUMELANINS;

intervention of cysteine
- gives rise preferentially to the production of CYSTEINYLDOPA isomers.
- Cysteinyldopas are then oxidized through redox reaction with dopaquinone to CYSTEINYLDOPAQUINONES that give rise to PHEOMELANINS

Cysteinyldopa formation is preferred over cyclodopa formation as long as cysteine concentration is higher than 0.13 µM

Dopachrome production becomes faster than cyclodopa production when the cyclodopa concentration is higher than 0.7 µM

Pheomelanogenesis is preferred over eumelanogenesis as long as cysteinyldopa concentration is higher than 9 µM

Tyrosinase activity is lower when pheomelanogenesis proceeds compared with eumelanogenesis

The switching to pheomelanogenesis is accompanied by a marked decrease in the melanin content of hair

THE PRODUCTION OF EU AND PHEOMELANINS IS UNDER CONTROL OF TWO INTERCELLULAR SIGNALING MOLECULES:

α – MSH (α – melanotropin)

ASP (Agouti signal protein)

α – MSH AND ASP HAVE ANTAGONISTIC ROLES AND POSSIBLY ANTAGONISTIC MECHANISMS OF ACTION IN MELANOCYTES.

α-MSH (melanotropin)

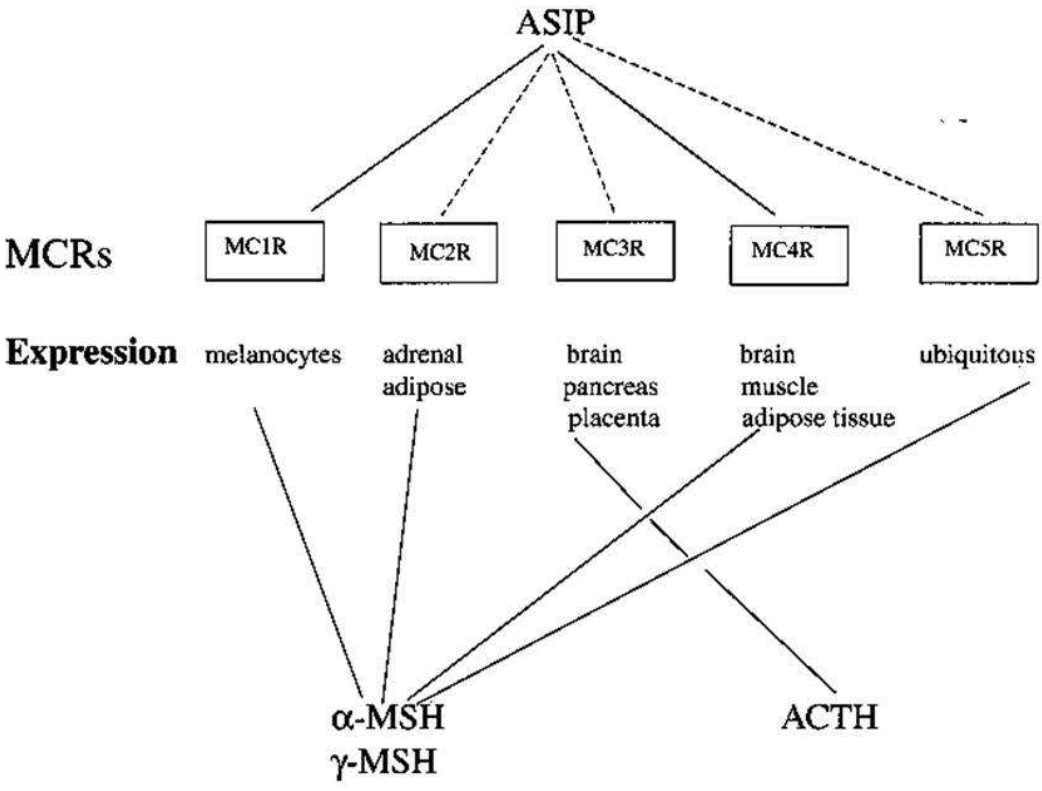
- Increases the synthesis of eumelanins in melanocytes
- Promotes the differentiation of melanoblasts into mature melanocytes

Encoded by the PROPIONMELANOCORTIN GENE (POMC)

Mechanism of action
bind to receptor that couple to heterotrimeric guanine nucleotide-binding proteins (G Proteins)

Activate adenylyn cyclase

RECEPTOR = Mc1r
MSHR mRNA expressed in melanocyte



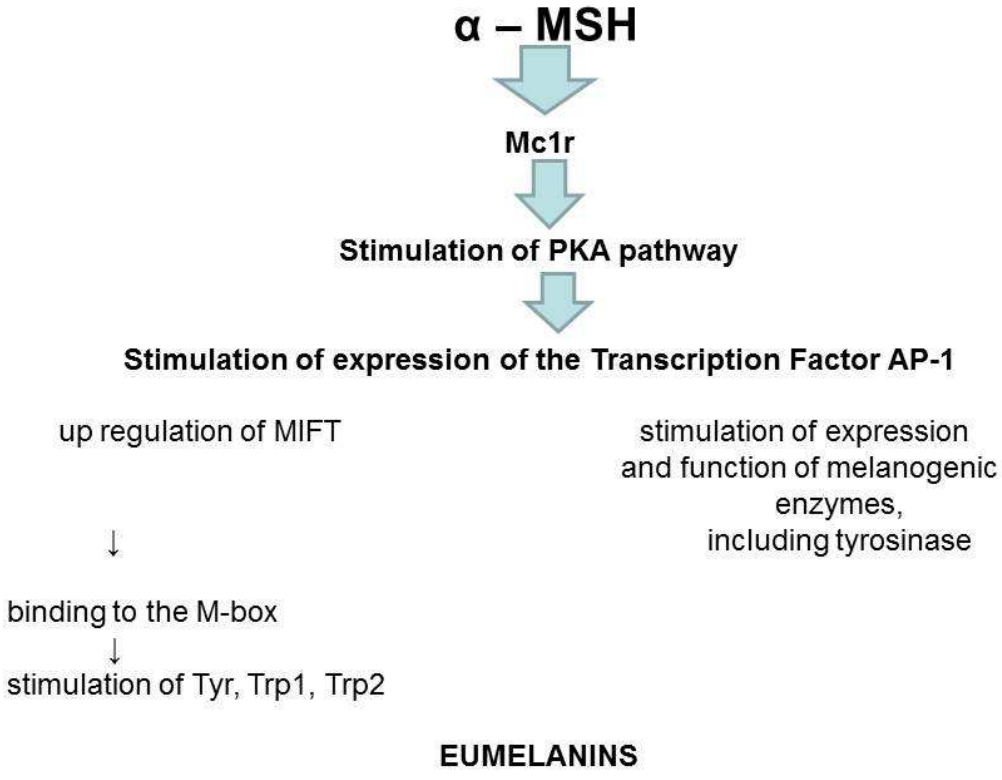
Mc1r (melanocortin-1-receptor)

MC1R (Extention) is a single exon gene encodes the G-protein coupled receptor (seven transmembrane protein), specifically expressed by melanocytes, that plays a crucial role in melanogenesis stimulation upon binding to its physiological ligand ASIP/α-MSH.

A functional *MC1R* allele can lead to eumelanin production depending upon which allele is present at the ASIP (Agouti) locus.

Gain-of-function Mc1r mutations cause exclusive production of eumelanins through the increased accumulation of cyclic adenosine monophosphate (cAMP)

Loss-of-function mutations cause exclusive production of pheomelanins



ASP (Agouti)

ASP (Agouti) **encodes for a small secreted factor (agouti signalling protein) that influence functions as a paracrine regulator of hair pigmentation.**

- **Produced by Dermal Papillae Cells**
- **Paracrine factor**

ASP regulates :

- melanoblast differentiation
- melanogenesis

Gain-of-function ASP mutations cause exclusive production of pheomelanins.

Loss-of-function mutations cause exclusive production of eumelanins.

Melanogenesis

ASP IS ANTAGONIST OF α – MSH SIGNALING MEDIATED BY THE Mc1r

Inhibit the α – MSH binding to the Mc1r

Acts through a receptor from the Mc1r

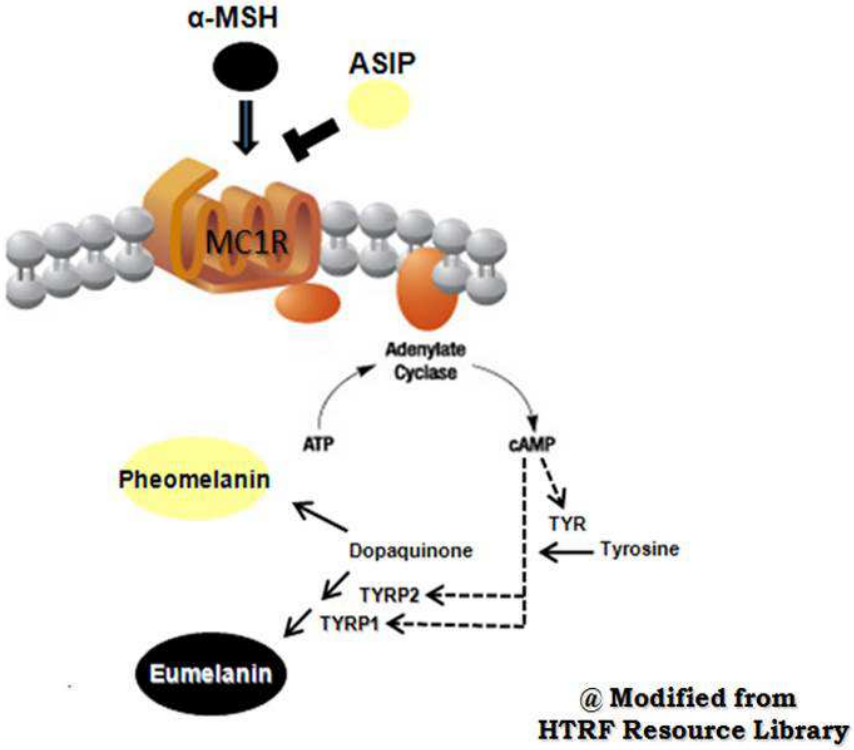
Decreases eumelanin synthesis

slight inhibition of tyrosinase activity

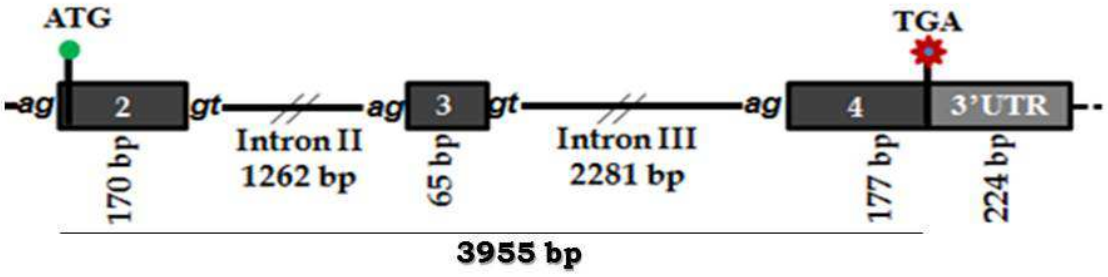
almost complete loss of Trp1 and Trp2 expression

Mc1r ASP INTERACTION

Mutually exclusive binding of the melanocortin-1-receptor (MC1R) by the agouti signalling protein or the α-melanocyte stimulating hormone (α-MSH) signals the hair-bulb melanocytes to synthesize either pheomelanin (yellow-red pigments) or eumelanin (dark pigments), respectively.

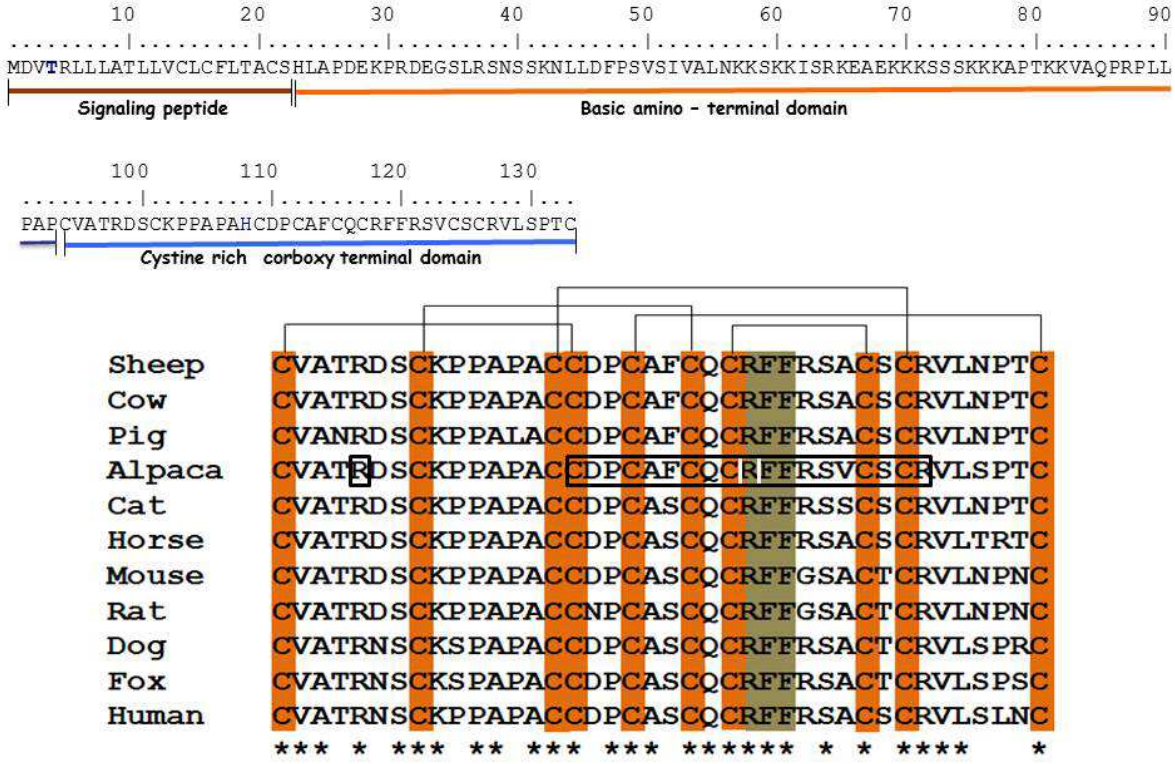


Structure of alpaca *agouti* genomic locus

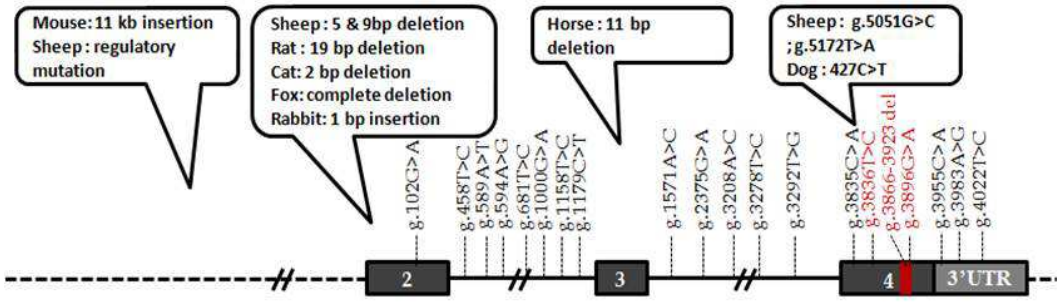


Organism name	GenBank accession number	Total length in base pair
Human	gi:224809234	8793
Rat	gi:62750360	6239
Sheep	gi:186703017	5191
Bovine	gi:1841897	5139
Pig	gi:60280616	4826
Rabbit	gi:164512185	4590
Dog	gi:74034289	4480
Horse	gi:14599451	4002

Alpaca agouti protein structure and mammalian agouti amino acid sequence alignment (cystine-rich domain)



Structure of alpaca *agouti* genomic locus and polymorphisms



In total – 20 SNPs

2- amino acid changing
g.3836T>C p.R98C (a^{hT})
g.3896G>A p.R118H (a^{H})

1- inframe deletion
g.3866-3923 del ($a^{\Delta 57}$)

LOSS-OF-FUNCTION MUTATIONS IN ASP

g.3836C>T (a^{hT}),

g.3881G>A (a^{H})),

in-frame 57 bp deletion (g.3866_3923del57) ($a^{\Delta 57bp}$)

in-frame 57 bp deletion (g.3866_3923del57) ($a^{\Delta 57bp}$)

- **114 amino acid containing agouti protein, which lacks 19 amino acids (p.C109_R127del19) from the cysteine (C) rich domain, which is critical in agouti function.**

g.3836C>T (a^{hT}),

- **change of arginine (R) to C, which would disrupt the highly conserved region of the protein.**
- ***a^{hT}* may produces agouti protein with minimal/partial activity.**

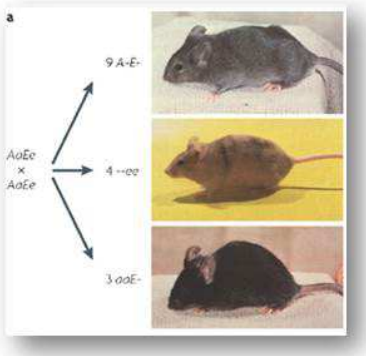
g.3881G>A (a^{H})

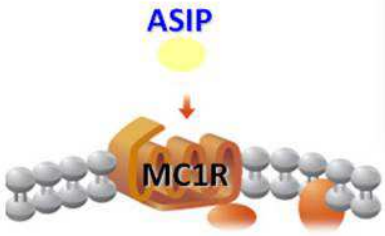
- **Change the R to histidine (H) in the cystine-rich domain, which disrupt the highly conserved Arg-Phe-Phe (R-F-F) motif in the protein**

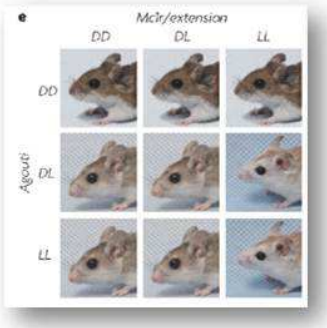
Agouti genotypes of the two _non-agouti_ mutations and an in frame deletion observed in exon-4 and allelic situation of the animals examined in the present study

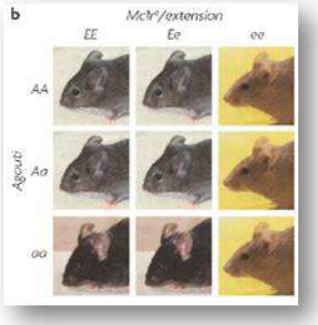
Phenotype	g.3836C>T p.R98C	g.3896G>A p.R118H	g.3866-3923del57 p.C109_R127del	Allele	Number of Animals
Black	C	A	-	a^H	17
	C	-	Yes	$a^{\Delta 57}$	
Black	C	A	-	a^H	10
	T	G	-	a^{hT}	
Brown	C	G	-	A	2
	C	G	-	A	
Brown	C	G	-	A	9
	T	G	-	a^{hT}	

Genetic interaction (Epistatic) of _agouti_ and _MC1R_









Dominant _agouti_ (_A_) induces Pheomelanin synthesis

Dominant _MC1R_ (_E_) induces Eumelanin synthesis

The *agouti* and *MC1R* genotypes in brown and black phenotypes

Genotype		Phenotype
***Agouti* (Ligand)**	***MC1R* (Receptor)**	
$a^{\Delta 57bp}/a^{H}$, a^{H}/a^{hT}	***E/E***, ***E/e***	Black
A/A, ***A*** $/a^{hT}$	***E/e***	Brown

BLACK ALPACA
Mc1r – ASP EPISTATIC INTERACTION

Loss-of-function ofis Agoiti mutation(s)

↓

Loss of ASP ability to block the ability of melanotropin (α – MSH) to activate Mc1r

↓

Mc1R is activadet
Wild ("normal") allele in Mc1r is expressed
High level and expression of MIFT
High expression of Tyr, Trp1 and Trp2

↓

Black eumelanogenesis

BROWN ALPACA
Mc1r – ASP NO EPISTATIC CODOMINANT INTERACTION

Normal ("wild") allele in both, ASP and Mc1r

↓

No epistatyc interaction between ASP and Mc1r

↓

Mixed melanogenesis

↓

Brown is the "normal" ("wild) phenotype in alpaca

BROWN IS THE "WILD" PHENOTYPE IN ALPACA FOR Mc1r AND ASP

BROWN IS DOMINANT ON BLACK PHENOTYPE

BLACK PHENOTYPE IS UNDER CONTROL OF AN ALLELIC HETEROGENEITY


THANK YOU ALL
CAMERINO