Mechanism of Melanin Deposition and Related Candidate Genes in Silkie Chickens: Postprint

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Abstract

The degree of black pigmentation in Silkie chickens is closely associated with their nutritional properties and medicinal value, representing one of the important economic traits of this breed. The black pigmentation of Silkie chickens is influenced by melanin deposition in the body. This article presents a comprehensive review of the mechanisms underlying melanogenesis and deposition, and discusses candidate genes that influence melanin deposition in Silkie chickens, with the aim of advancing understanding and research into melanin traits and related molecular breeding.

Full Text

Melanin Deposition Mechanism and Related Candidate Genes in Silky Fowls

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Abstract: The blackness of silky fowls is closely related to their nutritional characteristics and medicinal value, representing one of the most important economic traits in these birds. The degree of blackness in silky fowls is influenced by melanin deposition in the body. This paper provides a comprehensive review of melanin synthesis and deposition mechanisms, and discusses candidate genes affecting melanin deposition in silky fowls, aiming to enhance understanding and research on melanin traits and related molecular breeding.

Key words: silky fowl; melanin; candidate gene

Silky fowls are highly favored by consumers due to their exceptional nutritional and medicinal value. Consumers typically judge the quality of silky fowls by the intensity of their blackness, which is determined by the degree of melanin deposition in the body. Research has also revealed that the nourishing effects of silky fowls are primarily based on their melanin content [1]. Beyond conventional economic traits, the blackness of silky fowls—namely, melanin deposition—represents a core objective in breeding programs. Melanin is synthesized by melanocytes and transported to extracellular locations, where it deposits in surrounding tissues. Vertebrate melanocytes originate from the neural crest; during early embryonic development, melanoblasts differentiate from neural crest cells, migrate to the somatic mesoderm, and enter the ectoderm to form epidermal melanocytes, enabling widespread distribution of melanocytes throughout the embryo [2]. Melanin deposition in silky fowls is a complex process regulated by multiple factors. This review summarizes the progress in research on melanin synthesis, deposition patterns, and candidate genes in silky fowls, providing a reference for future studies.

1. Melanoblast Differentiation and Migration

During the neural system formation stage of early gastrulation, neural crest cells migrate along the dorsal pathway through somites and ectoderm, differentiating into melanoblasts that ultimately enter the ectoderm and form melanocytes in the dermis and even epidermis. Subsequent research discovered that neural crest stem cells can also differentiate into Schwann cell precursors, which migrate ventrally toward the skin and eventually develop into melanocytes [3-4]. In vitro culture of avian trunk neural cells demonstrated that neural crest cells with melanogenic potential begin migrating only after 6 hours [5]. In chicken embryos, neural crest cells initiate migration at developmental stages 12-13, and melanoblasts commence dorsal migration from the neural crest at stage 20 [6]. The migration pathways and differentiation trends of neural crest cells are regulated by various factors, subsequently affecting melanocyte generation and tissue distribution. Certain factors exhibit inhibitory effects on melanocyte formation and migration. FoxD3, a member of the fork head box (Fox) family and a key stem cell transcription factor, regulates neural crest cell generation, migration, and differentiation. Studies have shown that ectopic expression of FoxD3 in the chicken neural tube inhibits neural crest formation and its derivatives [7], and FoxD3 can also suppress melanoblast formation. Kos et al. [8] reported that FoxD3 inhibits neural crest cell differentiation into melanoblasts and suppresses melanoblast migration.

Conversely, melanocyte generation and migration are promoted by other factors. Microphthalmia transcription factor (Mitf) regulates melanoblast differentiation and development, serving as a marker for neural crest cell commitment to the melanocyte lineage. Research indicates that Mitf recognizes highly conserved E-box or M-box sequences in target gene promoter regions through its basic helix-loop-helix leucine zipper (bHLHZip) structure, initiating downstream tar-

get gene transcription to participate in melanocyte differentiation and development [9]. The paired box 3 (Pax3) transcription factor also plays crucial regulatory roles in melanocyte migration, differentiation, and anti-apoptosis. Neural cell adhesion molecule 1 (NCAM1) primarily promotes cell-cell adhesion, while Pax3 can directly inhibit NCAM1 expression or indirectly cause rapid phosphorylation of NCAM1 after synthesis, thereby losing adhesion function and promoting cell migration [10-11]. Pax3 synergizes with SRY-box containing gene 10 (Sox10) to promote Mitf expression, driving melanocyte differentiation. During embryogenesis, Pax3 suppresses p53-dependent apoptosis to ensure melanocyte development completion [12].

Melanoblast differentiation and migration primarily occur during embryonic stages, and the distribution of melanocytes in animal bodies largely determines the amount of melanin deposition. Tian et al. [13] observed through sectioning and staining of Taihe silky fowl skin and muscle tissues that melanocyte density was greater in the dermis than epidermis in skin tissue, while in muscle tissue, melanocytes were mainly distributed in the epimysium and some perimysium, with fewer in muscle fibers, resulting in darker outer surfaces than inner regions of pectoral muscles. Jiang Ming [14] reported similar findings in Guangxi silky fowls. Variations in blackness among individual silky fowls and differences in melanin deposition across tissues likely result from differences in melanocyte numbers. Numerous factors influence melanocyte numbers in the body, offering substantial potential for selection. However, reports on melanocyte proliferation, aging, and distribution patterns across different growth stages in silky fowl tissues are lacking, necessitating in-depth research to identify key molecular markers affecting melanocyte distribution and accelerate breeding progress for blackness traits.

2.1 Synthesis

Melanosomes are specialized organelles for melanin synthesis and storage in mature melanocytes, including eumelanosomes that produce black and brown pigments and pheomelanosomes that produce red, yellow, and brown pigments. Tyrosine (Tyr) is the primary precursor for melanin synthesis. Under the action of tyrosinase (TYR), tyrosine is catalyzed to form dopaquinone, which undergoes a series of oxidation and polymerization reactions to form dopachrome. Tyrosinase-related protein 2 (TYRP2) then hydroxylates dopachrome to 5,6-dihydroxyindole-2-carboxylic acid (DHICA). DHICA has two reaction pathways: one involves TYRP1-catalyzed conversion to 5,6-indolequinone carboxylic acid, a moderately soluble brown substance; the other involves decarboxylation to 5,6-dihydroxyindole (DHI), which is rapidly oxidized and polymerized under TYR catalysis to form 5,6-indolequinone, a high-molecular-weight soluble dark brown or black polymer. Both 5,6-indolequinone carboxylic acid and 5,6-indolequinone constitute eumelanin. Pheomelanin is a sulfur-containing pigment whose synthesis differs from eumelanin, involving cysteine (Cys) or glutathione (GSH) that combine with dopaquinone through a series of reactions to generate pheome-

lanin. The melanin generation process is illustrated in Figure 1 [Figure 1: see original paper][15].

Melanin synthesis involves a complex series of reactions regulated by multiple signaling molecules. TYR is the first rate-limiting enzyme in melanin synthesis, with its activity determining the speed and yield of melanin production. Low TYR activity leads to excessive GSH participation in melanin synthesis, forming large amounts of pheomelanin. TYRP1 and TYRP2 share 40% homology with TYR and play important functional roles in melanin synthesis [16]. The adenylate cyclase (AC) pathway is a crucial signaling pathway in melanin synthesis, where -melanocyte stimulating hormone (-MSH) binds to melanocortin-1 receptor (MC1R) to activate the AC pathway, increasing cyclic adenosine monophosphate (cAMP) content and subsequently enhancing melanocyte TYR activity to promote melanin synthesis [17-18]. Agouti signaling protein (ASIP) acts as an MC1R antagonist, competitively binding to MC1R with -MSH, leading to decreased cAMP content, reduced TYR activity, and increased pheomelanin production [19-20]. Mitf can also bind to promoters of TYR, TYRP1, and TYRP2 genes, stimulating their expression and promoting melanin synthesis. The blackness of silky fowls is determined by the ratio of eumelanin to pheomelanin, indicating that increasing TYR, TYRP1, and TYRP2 activities can promote eumelanin production and enhance blackness in silky fowls.

2.2 Transport

Melanocytes form a melanin unit by contacting over 40 surrounding keratinocytes through their dendrites and cell-cell structures, delivering melanin to these cells. Melanin transport essentially involves melanosome transfer, including intracellular and intercellular transport. Intracellular transport refers to the movement of melanosomes from the perinuclear region along dendrites to peripheral active zones, fixing melanin near the cell membrane —a process requiring coordination between microtubules, microfilaments, and motor proteins [21]. Microfilament motor proteins must first anchor to melanosomes; myosin utilizes energy generated from ATP hydrolysis to bind microfilaments and move short distances toward their barbed ends. A complex structure formed by GTP-binding protein (RAB27A), myosin 5a (MYO5A), and melanophilin (MLPH) connects myosin and melanosomes, facilitating melanosome transport [22-24]. Studies in mice with mutations in these three genes revealed melanosome aggregation in the perinuclear region, preventing transport to the cell membrane [25]. Only after reaching the cell membrane can melanosomes be transferred to surrounding keratinocytes. Research on the morphology and mechanisms of intercellular melanosome transfer is limited, with three possible pathways proposed: (1) basal keratinocytes acquire melanin by phagocytosing melanocyte dendritic tips; (2) melanocytes secrete melanin for keratinocyte phagocytosis; and (3) the two cell types fuse to form a common transport channel [26-27]. Reports suggest that melanosome transfer mechanisms are not singular but rather coexist through multiple pathways

[28]. Numerous studies indicate that defects in melanin transport mechanisms constitute a primary cause of diluted skin and feather pigmentation [29-31].

Xu et al. [32] investigated the "five-gray"phenotype in Anyi tile-like gray chickens, characterized by gray feathers, feet, skin, beak, and comb, with gray muscle color and periosteum. Their study revealed that this phenotype primarily results from MLPH gene mutations causing melanin transport defects. The intensity of melanin in silky fowl skin and muscle directly affects product quality, yet reports on the impact of melanin transport on melanin deposition in silky fowl skin and muscle remain scarce, warranting further investigation.

3.1 TYR Gene Family

TYR, TYRP1, TYRP2, and pre-melanosomal protein 17 (Pmel17) constitute the four members of the TYR gene family, all derived from a common ancestral gene. Studies have found that TYR gene expression is significantly higher in black feathers than white feathers in poultry [33-34]. The chicken TYR gene is a multiple allele located on the long arm of chromosome 1, band 4 region 4 [35]. Zheng et al. [36] reported that in white-down silky fowls, TYR gene expression levels in skin, muscle, and internal organs showed significant positive correlation with melanin content, indicating that TYR overexpression facilitates melanin deposition. The TYR gene possesses abundant variation resources in Chinese local chicken breeds, particularly silky fowls. Chen et al. [37] identified three single nucleotide polymorphism (SNP) sites in the -641 to -2,125 bp upstream regulatory region of the chicken TYR gene, which were significantly associated with shank and skin color.

TYRP1-encoded protein is a transmembrane glycoprotein synthesized in the endoplasmic reticulum and transported to melanocyte interiors. In human studies, TYR gene expression showed no significant difference between melanocytes of dark and light skin types, while TYRP1 gene expression was markedly elevated in dark skin [38]. Li et al. [39] found TYRP1 expression was 6.54-fold higher in black horses than gray horses. Liu Wei [40] discovered that in silky fowl embryos, the timing of visible melanin in tissues was later than TYRP1 expression onset, demonstrating TYRP1' s crucial role in melanin synthesis. TYRP2, also known as dopachrome tautomerase (DT), converts dopachrome to DHICA, controlling the DHICA/DHI ratio in melanocytes and accelerating eumelanin synthesis, representing an important protein affecting animal coat color [41]. Pmel17-encoded protein shares remarkable structural similarity with proteins encoded by the other three TYR family members, thus considered the fourth family member, participating in regulation of the terminal steps of melanin production. Pmel17 is a type I transmembrane glycoprotein synthesized in the endoplasmic reticulum, transported to melanosomes, and processed by proprotein convertases and proteolytic cleavage to form amyloid fibril structures that promote melanosome structure formation [42-43], facilitating melanin synthesis and deposition. The Pmel17 gene contains 11 exons. Studies have shown that a stop codon mutation [44] and a single nucleotide insertion causing an

additional 12 amino acids [45] in mouse Pmel17 produce silver coat phenotypes. A p.Arg618Cys mutation in horse Pmel17 also yields silver coat [46], while chicken Pmel17 p.R618C mutation produces reddish-brown feather phenotype, and a 9 bp insertion in exon 10 generates dominant white feather phenotype [47]. Pmel17 mutations affect melanosome maturation, causing melanin synthesis and deposition defects. Reports on Pmel17 gene effects on melanin deposition in silky fowl skin and muscle remain unreported, making further investigation crucial for understanding melanin deposition mechanisms.

3.2 MC1R Gene

The chicken MC1R gene is encoded by the extended black (E) locus and serves as a key regulator of melanin synthesis [48]. MC1R is a G protein-coupled receptor on melanocyte surfaces with seven transmembrane domains, functioning as the smallest G protein-coupled receptor that acts as an -MSH receptor in the AC pathway, playing a vital role in melanin synthesis [49]. Zheng et al. [33] investigated MC1R gene expression differences between white and black feathers in mule ducks and Muscovy ducks, showing 9.08-fold higher expression in black versus white feathers of mule ducks and 3.13-fold higher in black versus white feathers of Muscovy ducks, with both differences reaching extremely significant levels. Du [50] examined MC1R gene expression in skin, liver, and feather pulp of black and gray-white Bian chickens of the same age, rearing environment, and nutritional status, finding significantly higher expression in all tissues of black Bian chickens. Yang et al. [51] detected three SNPs in the chicken MC1R coding region significantly associated with skin, meat, and shank color. Studies have shown that E92K and M71T mutations in chicken MC1R produce completely black feathers, while H215P mutation inhibits melanin deposition [52]. Chi et al. [53] cloned the MC1R gene from silky fowls using reverse transcription PCR, revealing a full-length 945 bp gene encoding 314 amino acids with four distinct mutation sites: M71T, E92K, S124G, and H215P. The S124G mutation was hypothesized to cause black skin, meat, and bone with pure white feathers in silky fowls, though the specific mechanism requires further investigation.

3.3 Mitf Gene

Mitf is a key regulatory gene in the melanin synthesis pathway, influencing melanocyte differentiation and development while directly regulating tyrosinase gene family expression to affect melanin production [54]. Zhu et al. [55] found significantly higher Mitf expression in brown versus white coat skin tissues of alpacas. In birds, Mitf expression correlates with feather color. Li et al. [56] reported high Mitf expression in black feather bulbs of ducks but almost no expression in white feather bulbs. Minvielle et al. [57] found that Mitf mutations cause silver-white plumage in homozygous Japanese quail. Wang et al. [58] also identified a base mutation at position 1,109 bp in the Mitf coding region highly associated with white feather traits in Zhedong white geese. Zheng et al. [59] cloned the Mitf gene cDNA sequence from white-down silky fowls, obtaining

a 1,431 bp length encoding 468 amino acids, and found significant positive correlation between Mitf gene expression and melanin content across different tissues, indicating that Mitf expression facilitates melanin deposition in silky fowls.

3.4 MLPH Gene

MLPH participates in mature melanosome transport in melanocytes, causing melanosome aggregation at melanocyte dendritic tips and regulating animal skin and coat color. MLPH gene mutations reduce pigmentation, producing diluted coat phenotypes. Numerous domestic and international studies have found that MLPH gene mutations in cats, dogs, rabbits, quail, and American mink cause lighter coat color and diluted phenotypes [30-31, 60-62]. Liu et al. [63] discovered tissue-specific expression of MLPH gene in 20-week-old Zhedong white goose females, with highest expression in pigmented eyes and lower expression in dorsal skin, abdominal skin, and foot webs, while no expression was detected in heart or liver. This expression pattern aligns with goose melanin pigmentation, further confirming MLPH' s involvement in melanin deposition.

4 Summary

Silky fowl is a unique local chicken breed in China whose primary economic and medicinal value lies in melanin deposition. Conventional breeding for melanin traits primarily relies on visual assessment of blackness intensity, yielding slow progress, making molecular marker-assisted selection essential. Extensive research on melanin deposition mechanisms in animal coats provides an important foundation; investigating genes related to melanin deposition in silky fowls holds significant importance. Future research should examine melanin traits from multiple perspectives: melanoblast generation and migration, melanin synthesis and transport, and potential melanin degradation in tissues. Elucidating the specific mechanisms of gene regulation of melanin deposition, identifying major genes and molecular markers affecting melanin deposition, and utilizing these markers for early selection of melanin traits in production will enhance blackness intensity and accelerate breeding progress in silky fowls.

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Note: Figure translations are in progress. See original paper for figures.