THE MOLECULAR UNDERPINNINGS OF PHEOMELANIN SYNTHESIS AND CYSTEINE STORAGE

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The most noticeable of human polymorphisms that best represent human diversity are the variations in skin, hair and eye color. These polymorphisms are a result of evolutionary pressures on human populations that have adapted to different latitudes. Therefore, human pigmentation is a complex adaptive trait that can be correlated with geographic and environmental variation.[1] Dark complexion, for example dark hair, eye and skin color seen in equatorial populations, is thought to represent the ancestral allele that has been maintained by environmental selective forces at lower latitudes, which are high-UVR regions, to protect from DNA damage[2]. The depigmentation observed in Northern European populations, recapitulated by light hair, eye and skin color, was positively selected in order to facilitate vitamin D synthesis at higher latitudes with lower-UVR. At the same time, a certain level of pigmentation is retained for photoprotection. However, melanocytes in hair, skin and eye are distinct cell populations. In the skin the melanocyte represents a specialized cell type in the basal layer of the epidermis and have their own regulatory circuits. These signaling pathways give rise to trait-specific variants which result in phenotypic combinations such as dark hair, light skin and blue eyes common in European populations[3,4]. Differences between dark-skinned African populations and light-skinned European populations include melanosome number, density and size.

Animal melanin can be classified into two types of pigment: eumelanin, black to brown pigment and pheomelanin, yellow to red pigment. Both, eu- and pheomelanosomes contain the enzyme tyrosinase which converts L-tyrosine to L-dihydroxy-phenylalanine (DOPA) and to L-dopaquinone in the first two steps of melanin synthesis. If sufficient amounts of L-cysteine are present within the melanosome, dopaquinone and L-cysteine will eventually produce pheomelanin. The synthesis of eumelanin requires two additional membrane-bound enzymes TYRP1 and DCT (TYRP2), which are exclusively present in eumelanosomes[5]. TYRP1 and DCT form a protein complex to assist in the stabilization of tyrosinase. Spontaneous reaction produce dopachrome form the eumelanin precursor dopaquinone. DCT assists in the formation of the eumelanin precursor DHICA (5,6-dihydroxyindole-2-carboxylic acid). In the absence of DCT dopachrome spontaneously forms DHI (5,6-dehydroxyindole)[5].

However, genetic mutations that result in alterations in the pigmentation of skin, hair and eye may also have pathological consequences, such as Oculocutaneaous albinism (OCA)

being an extreme example[6]. OCA is a group of autosomal recessive disorders that concerns melanin synthesis and melanosome biogenesis. Its' pathology is mainly characterized by hypopigmentation, meaning partial or complete loss in pigmentation of hair, skin and eye. The reduction in pigmentation of the iris leads to impaired vision, due to iris translucency, reduced pigmentation of the retinal epithelium, congenital nystagmus, foveal hypoplasia and misrouting of the optic nerves. The prevalence of albinism has been estimated 1:17,000 worldwide, meaning that one in 70 people carry an OCA allele[7,8]. Over the past years many of the genes underpinning normal and pathological pigmentation phenotypes have been identified. On the basis of population genetics even more loci that contribute to variations in pigmentation could be identified[9]. Mutations in TYR (OCA1), OCA2, TYRP1 (OCA3), SLC45A2 (OCA4), SLC24A5 (OCA6) and C10orf11 (OCA7) are mainly responsible for albinism pathologies, whereas polymorphisms in most of these genes account for pigment variation within and between European, African, and Asian populations. The molecular roles of these genes associated with pigmentation are well understood. Among these genes are enzymes directly involved in pigment synthesis, proteins that regulate the development and differentiation of the pigment producing melanocyte and components of signaling pathways that directly and indirectly influence pigment synthesis[10,11]. While these genes belong to the best studied genomic loci, some pigmentation genes encode putative transport proteins. These putative transport proteins have been shown to localize to the melanosome membrane, a specialized lysosome-like organelle within the melanocyte which is the site for the synthesis and storage of the pigment melanin. It has been shown that these melanosomal membrane proteins likely regulate the availability of metabolites required for melanin synthesis and/or control melanosomal pH. While these genes represent some of the best studied loci in human genetics, the biochemical function of the proteins they encode remains elusive[1,12]. To date, it is unclear which known melanosomal metabolites could be their substrates. Apart from the key melanin synthesis intermediates and pH, little is known about the melanosomal metabolome. In order to fully understand the biology and pathology of pigmentation, it is crucial to elucidate the molecular function of these putative transporters as well as their physiological substrates. Furthermore, this knowledge could lead to new discoveries concerning ways how to modulate pigmentation and treat pigmentation pathologies.

In this project, we aim to understand the metabolic requirements of melanogenesis, especially those requirements upstream of physiological and pathological pigmentation variation. Towards this goal, this project is set out to characterize the melanosomal metabolome and to elucidate the function of putative pigment-related transport proteins.

The Sabatini lab has developed procedures to rapidly purify mitochondria and lysosomes followed by profiling and quantification their organellar metabolomes using LC/MS. Furthermore, organellar purification protocols have been proven to be useful in uncovering the function of transporters with unknown function. Organellar purification protocols have been proven to be useful in uncovering the function of transporters with unknown function. We leveraged our expertise in organellar purification, metabolite profiling and transporter biology and developed a novel procedure to rapidly isolate highly pure and intact melanosomes followed by metabolite extraction for LC/MS profiling in less than 12 min. we showed that we are able to capture melanosome specific metabolites. We have data that provide prove that we are able to capture melanosome specific metabolites. Among these melanosome specific metabolites are melanin synthesis intermediates such as indole-5,6-quinone, dopachrome and tyrosine. Due to the rapid immunopurification protocol we avoid loss of labile and unstable metabolites and we were able to expand our understanding of the metabolic requirements of melanogenesis. Furthermore, we were able to identify the substrate of melanosomal transporters and are working on understanding its role in melanin synthesis, pigment variation and pathology, which verifies the strength of our novel immunopurification approach as an invaluable tool for discovery.

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