Minireview

Tyrosinase and related proteins in mammalian pigmentation

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Abstract Tyrosinase is the key enzyme in pigment synthesis, initiating a cascade of reactions which convert the amino acid tyrosine to the melanin biopolymer. Two other tyrosinase-related proteins (TRP) are known, TRP-1 (probably DHICAoxidase) and TRP-2 (DOPAchrome tautomerase). These proteins show about 40% homology, and recent results have indicated that the genes might be derived from a common ancestor. We will discuss recent findings on genomic organization, and on the proteins and their presumed function, which is important for eumelanin synthesis in mouse and man.

Key words: Human; Mouse; Pigmentation; TRP-1; TRP-2; Tyrosinase

1. Introduction

Visible pigmentation in mammals results from the synthesis and distribution of melanin in skin, hair bulbs and eyes. At the cellular level, melanins are produced in pigment cells (melanocytes) in a specialized cytoplasmic organelle: the melanosome. Two types of melanins are synthesized: pheomelanins (yellow, red) and eumelanins (brown, black). Tyrosinase is essential in this synthesis, and catalyses 3 different reactions (Fig. 1). Two tyrosinase-related proteins (TRP-1 and TRP-2) have been characterized and attributed to specific reactions in melanin synthesis (Fig. 1). All 3 proteins are related and have about 40% amino acid identity with each other [1,2].

The genomic organization of the three genes is known in both mouse and man, and encompasses between 20 and 60 kb [3–5]. The coding region of 1.6 kb is scattered between 5 exons (tyrosinase), 7 exons (TRP-1) and 8 exons (TRP-2). According to the conservation of amino acids, corresponding codons can be identified between the three genes. Although the organization of exons and introns is divergent, some positions are nevertheless identical, thus suggesting a common ancestral gene (Fig. 2) [3,4]. Sequences of the promoter regions were compared with identify conserved regulatory elements involved in pigment cell-specific gene expression. An 11-bp consensus element, the so-called M box, was first identified in the promoters of mouse and human tyrosinase and TRP-1 and is also present in the TRP-2 promoter [3,6,7]. The product of the microphthalmia gene, a transcription factor of the basic helix-loop-helix family, can bind to the M box and is able to activate transcription of tyrosinase and TRP-1 in cell culture [8,9].

All the three proteins contain a signal peptide, a transmembrane domain, and histidine- and cysteine-rich sequences (Fig. 2). They might interact and participate in a multimeric complex of 200–700 kDa [10] which is possibly mediated by the cystein-rich sequences described as EGF repeat [11]. The histidine-rich regions are regarded as copper-binding sites in tyrosinase [12,13] and are, therefore, presumably implicated in the metal-binding capacities of the other TRPs.

The study of the TRPs is not only interesting in the process of pigmentation but they are also studied for recognizing and fighting melanoma: by their antigenic properties [14–16], as an early marker for metastases [17], and by the specificity of their promoter that can be used for gene therapy [18].

2. Tyrosinase (EC1.14.18.1)

The dramatic lack of pigment produced by *c* (*albino/Tyr*) locus mutations had suggested for a long time an implication of tyrosinase. In mice carrying deletions at the *c* (*Tyr*) locus, the tyrosinase gene is absent [5]. Introducing the gene into cells and back into albino mice induced pigmentation, thus, providing the ultimate proof that tyrosinase is encoded at the *c* (*Tyr*) locus [19,20]. The gene maps to mouse chromosome 7 and to human chromosome 11 [21]. The tyrosinase gene is composed of 5 exons and 4 introns and spans about 60–70 kb in mouse. The processed mRNAs are approximatively 2.4 kb in length but splicing is not always accurate and misspliced mRNAs are generated and may represent more than 10% of the tyrosinase transcripts [5]. These transcripts cannot be translated into catalytically competent proteins, but nothing is known about their function. Tyrosinase is synthesized as a 55-kDa protein with an isoelectric point of about 4.2. Following glycosylation, the molecular weight of the mature protein shifts to about 65–75 kDa (or even up to 80 kDa) [15,21].

Tyrosinase catalyses the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone (Fig. 1) [21,22]. DOPA is also a cofactor for the first enzymatic reaction, probably interacting with the oxidative state of the copper ion [23]. A third enzymatic role has been proposed for tyrosinase: the oxidation of 5,6-dihydroxyindole to 5,6-dihydroxyquinone [22,24]. However, it has also been proposed that this oxidation is performed through a peroxidase [25]. Tyrosinase preparations are enzymatically active at a pH range of 5–8, but in vivo the enzyme might encounter even lower values due to the acidic intramelanosomal pH [26]. In human, many mutations in the tyrosinase gene have been described and are responsible for oculo-

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Abbreviations: TRP, tyrosinase-related protein; DOPA, 3,4-dihydroxyphenylalanine; DHI, 5,6-dihydroxyindole; DHICA, 5,6-dihydroxyindole-5-carboxylic acid; Dct, DOPAchrome tautomerase; EGF, epidermal growth factor; MSH, melanocyte-stimulating hormone.

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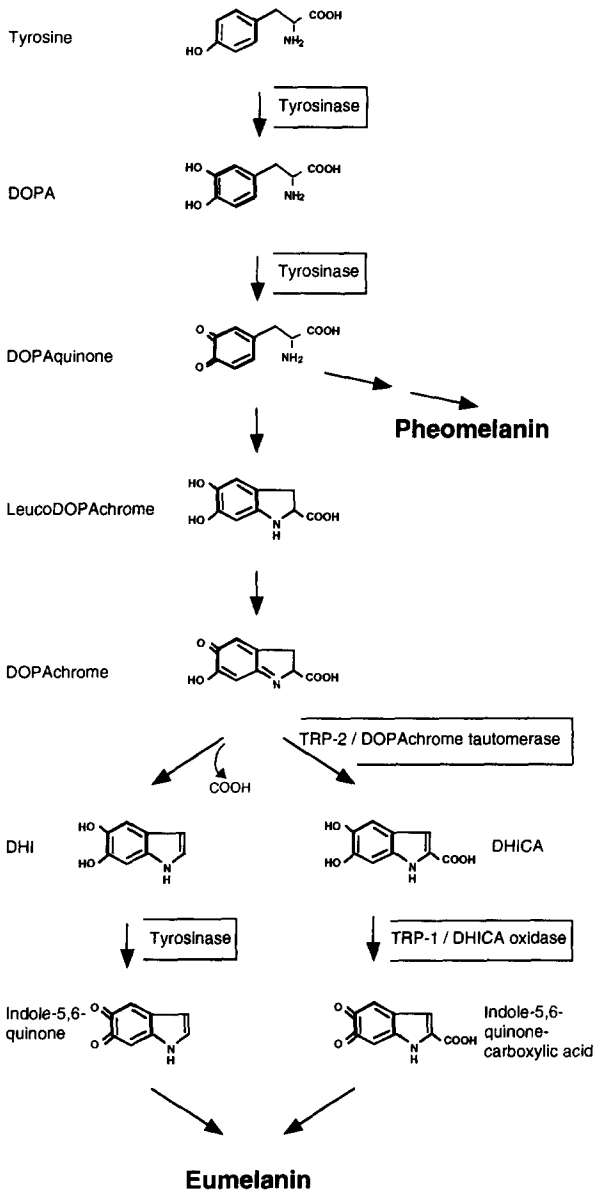

Fig. 1. The melanogenic pathway from tyrosine [33,38]. Enzymatic reactions attributed to tyrosinase, TRP-1 and TRP-2 are indicated. DOPA, 3,4-dihydroxyphenylalanine; DHICA, 5,6-dihydroxyindole-2-carboxylic acid; DHI, 5,6-dihydroxyindole.

cutaneous albinism (type I). The study of these mutations unveiled the important catalytic regions of tyrosinase particularly in the histidine-rich regions that can complex copper [27].

3. Tyrosinase-related protein-1

The first cloned pigmentation gene was later mapped to the mouse *brown* (*b*/*Tyrp*) locus, and the protein was named tyrosinase-related protein-1, due to its homology to tyrosinase [28]. The gene is located on mouse chromosome 4 and on human chromosome 9 [29]. The TRP-1 gene encompasses 8 exons separated by 7 introns, including the first non-translated exon (Fig. 2; [3,4]). The coding sequence itself is 1.6 kb long, yielding a protein of 537 amino acids, including the signal peptide. The mature glycosylated protein has a molecular weight of about 75 kDa [21,30]. Between human and mouse, a high degree of sequence identity is existing in coding sequence (>90%) and the promoter (up to 80%) [3,4,6].

The function of TRP-1 is subject of much controversy, and it was proposed that TRP-1 shows activity of tyrosinase [31], represents another DOPAchrome tautomerase [32] or a melanosome-specific catalase [30]. Recent experiments on the mouse TRP-1 provided evidence that the protein functions as DHICAoxidase, a reaction downstream in the melanin synthetic pathway (Fig. 1, [31,33]).

4. Tyrosinase-related protein-2/DOPAchrome tautomerase (EC 5.3.2.3)

The enzyme DOPAchrome tautomerase is involved in the conversion of the red compound DOPAchrome to a colorless eumelanic intermediate which has been identified as 5,6-dihydroxyindole-5-carboxylic acid (DHICA), and was named successively L-DOPAchrome conversion factor [34], L-DOPA-

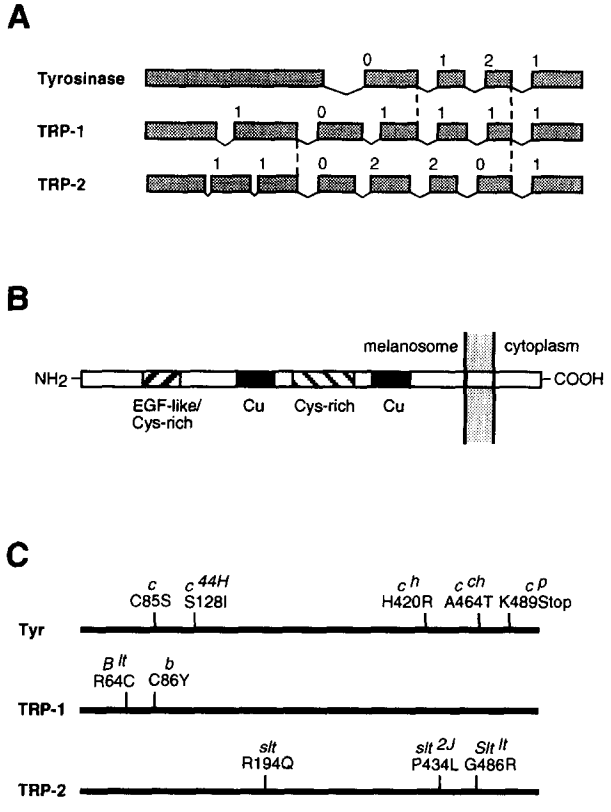

Fig. 2. The tyrosinase family. (A) Comparison of exon/intron structure of tyrosinase, TRP-1 and TRP-2, as identified in human and mouse [3,4]. Non-coding parts of the mRNAs are not indicated, as the non-coding exon of TRP-1 [6]. Interruptions of phases within introns are indicated by numbers, between codons (=0), and leaving either one base (=1) or two bases (=2) of a codon on the 5′ part of an intron. Identical positions of introns are indicated. (B) Schematic representation of the tyrosinase family protein domains, with the N-terminus at the left, inside the melanosome, and the C-terminus at the right, in the cytoplasm [3,4,47]. The N-terminal signal peptide is not depicted. Cys, cysteine; Cu, copper(metal)-binding sites (histidine-rich). (C) Scheme of the identified mouse mutations of the tyrosinase family proteins and their molecular defect. The *albino* (*c*) and *brown* (*b*) mutation lead to complete loss of enzymatic activity. The other characterized mutations affect pigmentation by changes in enzymatic activity [1–3,47,48]. c^{44H}, dark-eyed albino; c^{h}, himalayan; c^{ch}, chinchilla; c^{p}, platinum; B^{lt}, light; *slt*, slaty; Slt^{lt}, slaty-light.

chrome oxidoreductase [35], and L-DOPAchrome isomerase [36]. The enzyme has finally been renamed DOPAchrome tautomerase (Dct), a name which describes more precisely the reactions involved [37].

In 1992, the molecular cloning of TRP-2 confirmed the existence of a third member of the tyrosinase-related protein family [11,38]. The coding sequences are 1.6 kb long and are distributed on 8 exons (Fig. 2) [3,4]. A common regulatory motif, the M box, has equally been identified in the regulatory regions of the mouse and human TRP-2 gene [3]. The gene maps to the *slaty* (*Tyrp2*) locus on mouse chromosome 14 and to human chromosome 13 [11,39]. The molecular weight of the fully processed protein is about 75 kDa [38].

5. TRPs and melanogenesis

Tyrosinase initiates melanogenesis, but different posttyrosinase factors interfere to modulate the quantity and quality of the pigment produced. DOPAquinone is the key intermediate in the formation of pheo- vs. eumelanins and different factors are involved in the type of melanin synthesized: sulfhydryl compound as precursor of cysteine, MSH (melanocyte-stimulating hormone) and TRP-1.

Cysteine interacts non-enzymatically with DOPAquinone to form cysteinylDOPA, which is regarded as direct precursor of pheomelanin. The kinetics of reaction between sulfhydryl compound and DOPAquinone is much more rapid than the cyclization of DOPAquinone into LeucoDOPAchrome [40], and, thus, the concentrations of cysteine are critical for the type of melanin synthetized.

In mice, mutations at the *e (extension/Mc1r)* locus (encoding melanocyte-stimulating hormone receptor) and the *a (agouti)* locus (encoding an antagonist of the hormone) modulate the pattern of melanogenesis affecting changes in coat color [1]. Very recently, it has been shown that variations in human MSH receptor (Mc-1r) gene were more frequent in red hair individuals than in individuals with black or brown hair [41]. Intracellulary, eumelanogenesis might be favored by MSH via modulation of tyrosinase, TRP-1 and TRP-2 (Dct) activity [42].

TRP-1 function and presence is not essential for pigmentation in mice but the synthesis of brown rather than black eumelanin in mutant mice (*brown* mutation) indicates an important step in the synthesis and regulation of eumelanogenesis. Moreover, in human cell lines, expression of TRP-1 was only detectable in cells containing eumelanin [43]. The DHICA oxidase activity described for TRP-1 implicates this enzyme in the synthesis of eumelanin. Therefore, the chemical analysis of the eumelanic polymer, synthesized in the presence of TRP-1, will be important for understanding phenotypic melanogenic differences.

Once DOPAchrome is obtained, TRP-2 (DOPAchrome tautomerase) or metal ions (as copper or zinc) favor the formation of DHICA instead of DHI [44]. It has been suggested that DHICA- and DHI-derived melanins differ in several properties, such as solubility, flocculence and color [45]. The physiological role of TRP-2 is not clear, and there may be mutations at the *slaty/Tyrp2* locus that abolish TRP-2 activity, such as slaty-light, but this is not known [3,11]. It is even possible that slaty and slaty-2J completely abolish TRP-2 activity, and that residual Dct activity is derived from other enzymes. In human, case studies of chromosomal deletions involving 13q31–q32 have not reported any apparent pigmentation abnormalities [46]. However, it is possible that TRP-2 protects the melanocyte against cytotoxicity of decarboxylated indolic melanogenic intermediates by limiting their formation.

Despite the considerable progress in characterizing the 3 genes involved in melanogenesis, several questions remain. It is not clear whether other enzymes are existing in the eumelanogenic pathway, or whether TRP-1 has other functions besides DHICAoxidase. Moreover, the pheomelanogenic pathway is largely unknown, and the structure of the final melanin polymer is not resolved and might, thus, contain both eumelanin and pheomelanin.

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