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SHORT COMMUNICATION

Indication of a common ancestry for copper tyrosinases and heme catalases revealed by hydrophobic cluster analysis of the brown locus protein sequence

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In mammalian melanocytes, a membrane-anchored high molecular weight protein complex consisting of tyrosinase (monophenol monooxygenase, EC 1.14.18.1) and two tyrosinase-related proteins (TRP-1 and TRP-2, respectively), catalyzes the conversion of tyrosine into the pigment eumelanin. These three proteins are members of a large gene family of binuclear copper proteins (Halaban and Moellmann, 1990). TRP-2 has recently been shown (Tsukamoto *et al.*, 1992; Solano *et al.*, 1996) to possess DOPAchrome tautomerase activity (EC 5.3.2.3). On the other hand, the function of TRP-1, the most abundant glycoprotein expressed in human melanocytes remains controversial. This protein, encoded by the brown locus, exhibits more than 50% sequence identity with tyrosinase (Cohen *et al.*, 1990) and shows some tyrosine hydroxylase activity, but may specifically act as 5,6-dihydroxyindole-2-carboxylic acid (DHICA) oxidase (Jimenez-Cervantes *et al.*, 1994; Kobayashi *et al.*, 1994). However, this protein has also been suggested to act as a melanocyte-specific catalase entitled TRP-1 or catalase B (Halaban and Moellmann, 1990). Several observations support this: the oxidation of 5,6-dihydroxyindole and DHICA is associated with the generation of hydrogen peroxide, which is in turn known to destroy melanin and some of its precursors. H_2O_2 may thus regulate the expression of tyrosinase in melanocytes (Karg *et al.*, 1993), and obviously the course of melanogenesis is strongly affected by factors modulating the level of intracellular peroxides (Nappi and Vass, 1996). Furthermore, H_2O_2 is a rather potent reversible inhibitor of tyrosinase (Schallreuther *et al.*, 1991), and the oxy-form of this enzyme even shows a limited catalatic potential (Wood and Schallreuther, 1991).

The evolutionary relationships among different members of the tyrosinase family have been studied (Morrison *et al.*, 1994), but so far there was no indication of any structural as well as evolutionary relatedness between TRP-1 (catalase B) and the family of heme containing catalases (EC 1.11.1.6). This is caused mainly by the fact that the sequences of brown locus proteins (which obviously bind copper) and those of heme catalases exhibit no easily detectable sequence similarities. For instance, the sequence identity between human TRP-1 (Cohen *et al.*, 1990) and *Saccharomyces cerevisiae* catalase A (Cohen *et al.*, 1988) is only about 16% in the 400 amino acid overlap (alignment not shown). The presumably related function of these two supposedly structurally unrelated enzymes has evoked the idea on their eventual very distant evolutionary relationship. We have recently shown that one of the most strictly conserved structural parts among heme catalases is formed by the major substrate channel allowing the diffusion of peroxides from the protein's surface to the active site (Zámocký *et al.*, 1995, 1997). If there are any correspondences between heme catalases and TRP-1 (eventual copper catalase), these will be most likely found in such essential substructures. Therefore our primary search was for sequences of TRP-1 corresponding to this partial sequence of the major substrate channel of heme catalases.

Due to the absence of any striking sequence similarities between TRP-1 and heme catalases, we applied the Hydrophobic Cluster Analysis (HCA) (Gaboriaud *et al.*, 1987), a sensitive method for analyzing distantly related amino acid sequences, to trace the correspondences (if any) between the sequences of these different proteins. Interestingly, as can be seen from Figure 1, the HCA plot for human TRP-1 contains the analogous narrow cluster preceded by a proline residue (Pro146 in the *S.cerevisiae* catalase A; unless otherwise specified, all amino acid numbering throughout the text corresponds to *S.cerevisiae* catalase A), the pattern of which is very characteristic of the HCA plot of most heme catalases (not shown). It corresponds to the lower part of their major substrate channel with two important, almost invariant phenylalanines (Zámocký *et al.*, 1997), Phe148 and Phe149. By comparing the primary sequences of human tyrosinase and human TRP-1 (alignment not shown) the corresponding part was traced in the HCA plot of tyrosinase. The tyrosinase residues corresponding to the above mentioned phenylalanines are Tyr148 and Val149 (Figure 1).

Analysis of the HCA plots of the entire amino acid sequences revealed further convincing similarities depicted in Figure 1. Remarkably, the two residues which function as iron ligands in heme catalases (His70 and Tyr355), are both present in TRP-1 (His81 and Tyr362). On the other hand, only the putative equivalent of Tyr355 can be traced in the tyrosinase sequence (Phe347) (Figure 1). The presence of the equivalents of the iron-ligands in the sequence of TRP-1 (catalase B) is of special importance, taking into account that TRP-1 should bind copper like tyrosinases. However, there is no clear trend of conservation for all other residues involved in heme binding by the yeast catalase A (M.J.Mate-Perez, M.Zámocký, C.Herzog, L.M.Nykyri, P.M.Alzari, C.Betzel, F.Koller and I.Fita, in preparation) in either TRP-1 or tyrosinase. This indicates that TRP-1 contains only residual features characteristic of the iron binding in the family of heme catalases. Furthermore, the several sequence identities between the heme catalase A and TRP-1 (e.g. Ser198 and Gly201, and Thr336, Pro338 and Ser343) that are only conservatively substituted in tyrosinase (Ala200 and Ala203, and Ser328, Ser330 and Ala335, respectively) may indicate that the TRP-1 is an intermediate protein between the two structurally and functionally different enzyme families of heme catalases and copper tyrosinases. This idea is substantiated by the higher degree of sequence similarity between human TRP-1 and yeast heme catalase A than between the human copper tyrosinase and catalase A. The function of a catalase and a structure of a tyrosinase, however, strongly suggest for the eventual evolutionary 'intermediarity' of TRP-1.

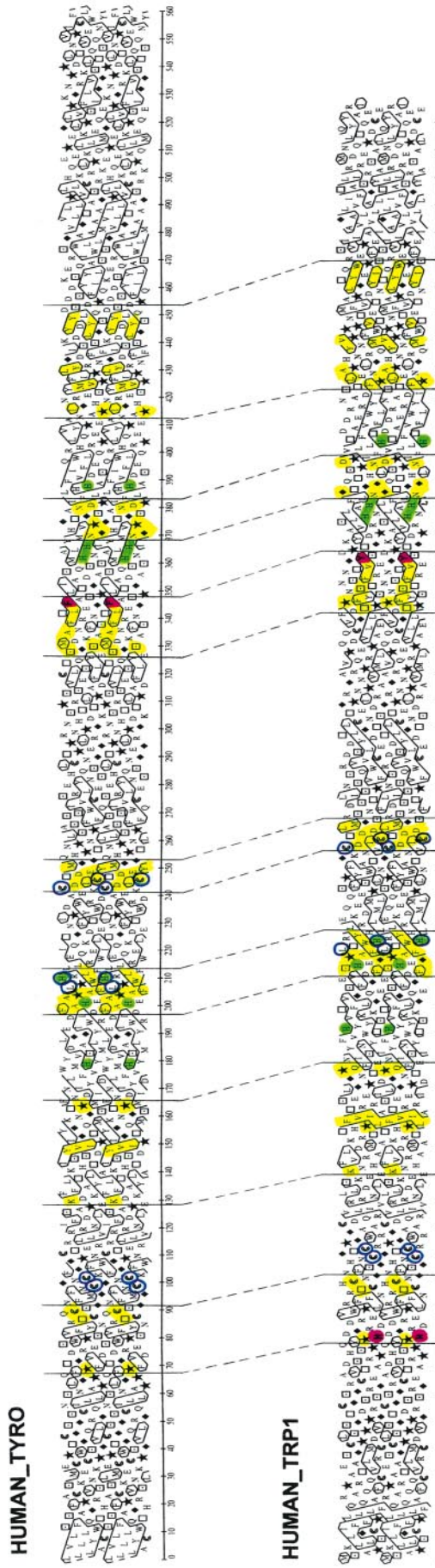

Fig. 1. HCA plots showing the correspondences between the families of copper tyrosinases and heme catalases. HUMAN_TYRO, human copper tyrosinase (Kwon *et al.*, 1987; GenBank J03581); HUMAN_TRP1, human TRP-1 (catalase B, brown locus protein, gp75) (Cohen *et al.*, 1990; X51420) structurally belonging to the tyrosinase family with function of a catalase; YEAST_CATA, heme catalase A from *Saccharomyces cerevisiae* (Cohen *et al.*, 1988; X13028). The regions in which the corresponding sequence similarities between the tyrosinase and catalase families can be found, are highlighted in yellow. The six histidines involved in the binding of copper in tyrosinase family enzymes (and the equivalent in catalase A) are depicted in green. The histidine and tyrosine residues essential for function of the heme catalase A (and the equivalents in tyrosinase and TRP-1) are depicted in magenta. The cysteines and leucine with histidine from the Cys-Xaa-Xaa-Cys and Leu-Xaa-Xaa-His motifs, respectively (discussed in the text), are signified by blue circles. The human TRP-1 is well-known to display a high degree of sequence similarity (about 50% identity) to human tyrosinase. The correspondences between these two proteins (easily detectable in their HCA plots) are therefore not highlighted here. In the HCA plots amino acid sequence of a protein is drawn as an unrolled and duplicated longitudal cut of a cylinder, where residues follow an α-helical pattern. Special symbols are used for glycine (◆), proline (★), cysteine (**C**), serine (□), and threonine (□). Clusters of hydrophobic residues are automatically contoured by the HCA-plot program. Analysis of the HCA plots was made following the published guidelines (Lemesle-Varloot *et al.*, 1990; Callebaut *et al.*, 1997).

Halaban and Moellmann (1990) have furthermore discussed that human TRP-1 and tyrosinase might accommodate bound heme, since both contain the sequence segments Cys-Xaa-Xaa-Cys and Leu-Xaa-Xaa-His, which are involved in heme binding by, for instance, cytochrome *c* and plant peroxidases (Welinder, 1985). It should be pointed out, however, that only one copy of the Cys-Xaa-Xaa-Cys motif is present in the fungal tyrosinases from *Neurospora crassa* (Kupper *et al.*, 1989) and *Aspergillus oryzae* (Fujita *et al.*, 1995), whereas it is present twice in the mammalian enzymes (99_CGNC and 243_CDIC; see Figure 1). furthermore, in contrast to the mammalian homologs, this motif in fungal tyrosinases is located close to the C-terminus of the protein (574_CGKC in *N.crassa* and 483_CANC in *A.oryzae* enzymes). The tyrosinases from the genus *Streptomyces* (Bernan *et al.*, 1985; Huber *et al.*, 1985) lack this motif completely. On the other hand, the second motif (comprising one of the copper-ligated histidines; 207_LPWH in human tyrosinase; see Figure 1) is absent in the fungal tyrosinases but unambiguously present in the prokaryotic enzymes from streptomycetes (60_LPWH in both *S.antibioticus* and *S.glaucescens* enzymes). Of the two Cys-Xaa-Xaa-Cys motifs and the one Leu-Xaa-Xaa-His present in the mammalian tyrosinase and TRP-1, the heme-binding catalase A contains only relicts of the motifs, i.e. one cysteine residue in the two possible Cys-Xaa-Xaa-Cys equivalent segments (Cys104 and Cys258, respectively) and the histidine corresponding to the one from the Leu-Xaa-Xaa-His motif (His210) is not preceded by leucine in the *i–3* position (Figure 1). This may reflect the very distant evolutionary ancestor common not only for copper tyrosinases and heme catalases but also for other heme-binding proteins. In these terms the other mammalian enzymes that metabolize tyrosine could be of interest, e.g., brain tyrosine hydroxylases. These enzymes, however, unlike tyrosinases do not belong to the binuclear copper proteins. They bind one single non-heme iron per subunit and exhibit substantial sequence similarities with phenylalanine hydroxylases and tryptophan hydroxylases to form a family of aromatic amino acid hydroxylases (Grenett *et al.*, 1987). This was recently confirmed by the crystal structures of the catalytic domains of tyrosine and phenylalanine hydroxylases (Erlandsen *et al.*, 1997; Goodwill *et al.*, 1997). The overall fold of both hydroxylases, however, reflects no similarity with typical heme catalases.

Interestingly, all sections showing structural relatedness (according to HCA in Figure 1) between catalase A and TRP-1 (catalase B), correspond to very distinct structural features of catalase A. In addition to the already mentioned substrate channel this includes parts of the central β-barrel domain, a critical β-turn of the so-called wrapping domain, and the part of the α-helical domain which docks to the β-domain. This presumably indicates that the structural relatedness of the two proteins is significantly more pronounced than could be expected from the respective primary structures. With regard to the eventual doubts concerning the ‘unusual’ evolutionary relatedness between animal enzymes (proteins) and microbial ones, that moreover share no common function, the case presented here of human copper tyrosinase and yeast heme catalase is not exceptional at all. Wells and Hediger (1992) have for instance observed the sequence similarity between the microbial enzymes from the α-amylase family on the one side and mammalian amino acid transport-related proteins and the so-called 4F2 heavy-chain cell surface antigens on the other side. We have recently demonstrated that the mammalian proteins may have recruited from the bacterial α-amylase family enzymes by the loss of catalytic activity and a part of the structural domain characteristic for the α-amylase family, respectively (Janeček *et al.*, 1997).

To explain the remarkable ‘hidden’ similarities between copper tyrosinase and heme catalase two basic possibilities are offered: (i) copper tyrosinases might have evolved from heme catalases via TRP-1 which has retained the catalase activity with the structure amended towards the features characteristic of the present-day tyrosinase family (or *vice versa*, TRP-1 having retained most of the structural features characteristic of tyrosinases with recruitment of catalatic function allowed by slightly changed structure); or (ii) both extant families, i.e. copper tyrosinases and heme catalases might have been the products of a protein ancestor represented nowadays by TRP-1.

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