The Tyrosinase Protein Families

Chem184

May 13, 2008

How can you identify a member?

Is protein sequence information enough?

Is catalytic activity enough?

What reaction(s) can tyrosinases catalyze?

What reaction(s) <u>do</u> tyrosinases catalyze *in vivo*?

Do all tyrosinases catalyze the same reaction(s)?

Agaris bisporus
White button, brown button, Portobello

Agaris bisporus Tyrosinase:

- isolated in 1858 (Shoenbein)
- purified in 1938 (Kibowitz)

Enzymatic Browning in Plants/Fungi

- Polyphenol Oxidases: Tyrosinase and Laccase
- Laccases
  - also a type 3 copper enzyme
  - activity against ortho- and para-phenols and are inactive against monophenols
  - Different pH optima than tyrosinase
  - Different susceptability to inhibitors
  - No significant laccase in *A. bisporus* (makes our life easier)
- Peroxidases
  - Oxygen from H_2O_2 not O_2

Homologs: Two genes descended from a common ancestor

- Two genes with high sequence identity/similarity are usually *assumed* to be homologs.
- Sequence similarity is **not** essential (hemocyanin and tyrosinase)
- Similar structure is pretty much essential (same general folds)
- Similar/Identical function is not essential (hemocyanin and tyrosinase)

Isoforms, isoenzymes, glycoforms, allozymes

ISOFORMS: most general term, same or different gene, different splicing, different proteolysis, different glycosylation, different phosphorylation

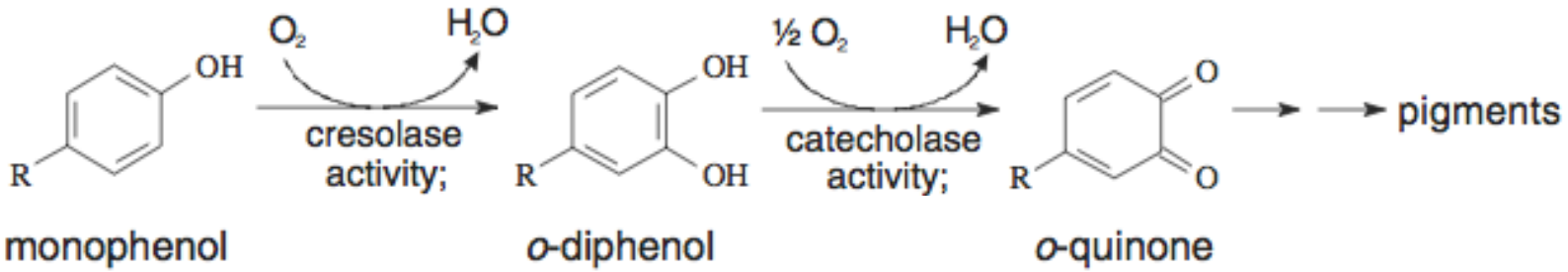
ISOENZYMES: different genes, same reaction (tend to have different K_M) *??*

GLYCOFORMS: different glycosylation pattern

ALLOZYMES: products of different allelles of the same gene

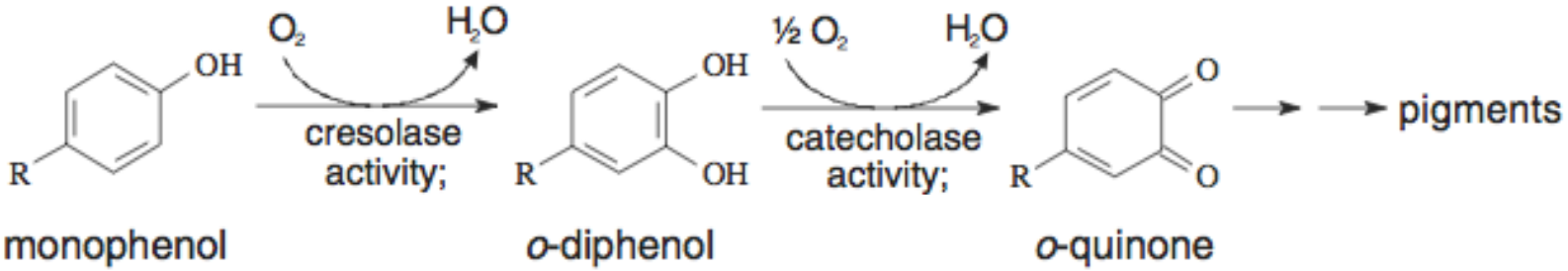
Tyrosinase

- Involved in the synthesis of melanins and other polyphenolic compounds.
- Able to (1) insert an oxygen in a position ortho- to an existing hydroxyl in an aromatic ring and (2) oxidizes the resulting diphenol to the quinone
- Molecular oxygen is used



hydroxylation of monophenol

oxidation of diphenol



hydroxylation of monophenol

oxidation of diphenol

CATECHOL OXIDASE ≠ TYROSINASE

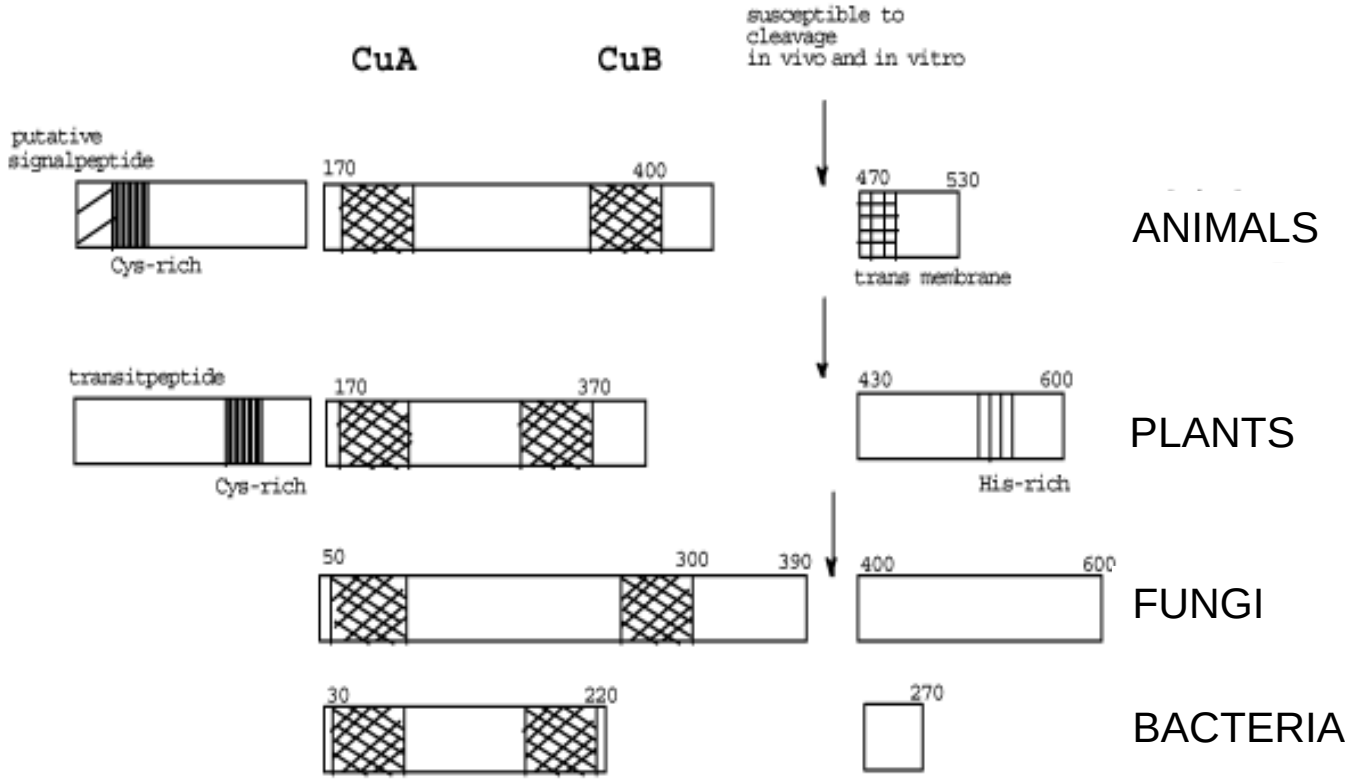
BUT SEE LATER …

Tyrosinase: Nearly ubiquitous

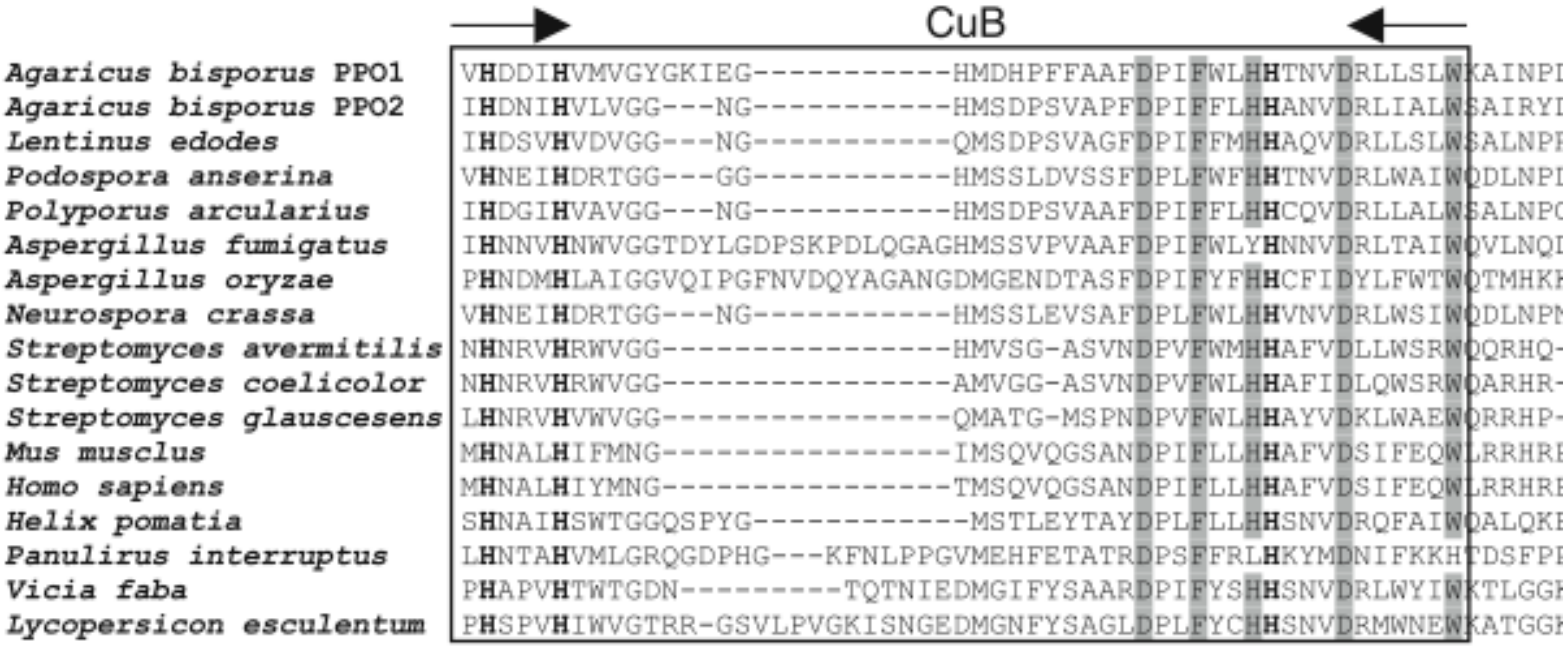
- Bacteria, plants, animals, fungi
- Higher similarity within groups than between
  - No common structure
  - Differ with respect to sequence (20%), primary structure, glycosylation pattern, activation characteristic

Similarities:

- **Binuclear type 3 copper center in active site**
- **All have 2 conserved regions CuA and CuB**


susceptible to
cleavage
in vivo and in vitro
CuA
CuB
putative
signalpeptide
170
400
470
530
Cys-rich
trans membrane
ANIMALS
transitpeptide
170
370
430
600
Cys-rich
His-rich
PLANTS
50
300
390
400
600
FUNGI
30
220
270
BACTERIA

tyrosinase	*Agaricus bisporus* PPO1 *Agaricus bisporus* PPO2	**White button mushroom (& portobello)**
	Lentinus edodes	**Shitake**
	Podospora anserina *Polyporus arcularius* *Aspergillus fumigatus* *Aspergillus oryzae* *Neurospora crassa*	**Other fungi**
	Streptomyces avermitilis *Streptomyces coelicolor* *Streptomyces glaucescens*	**Streptomyces (gram + soily bacteria; neosporin)**
	Mus musculus *Homo sapiens*	**Mouse** **~~Human~~**
haemocyanin	*Helix pomatia* *Panulirus interruptus*	**Snail** **~~Spiny lobster~~**
catecholase	*Vicia faba* *Lycopersicon esculentum*	**Fava bean** **~~Tomato~~**

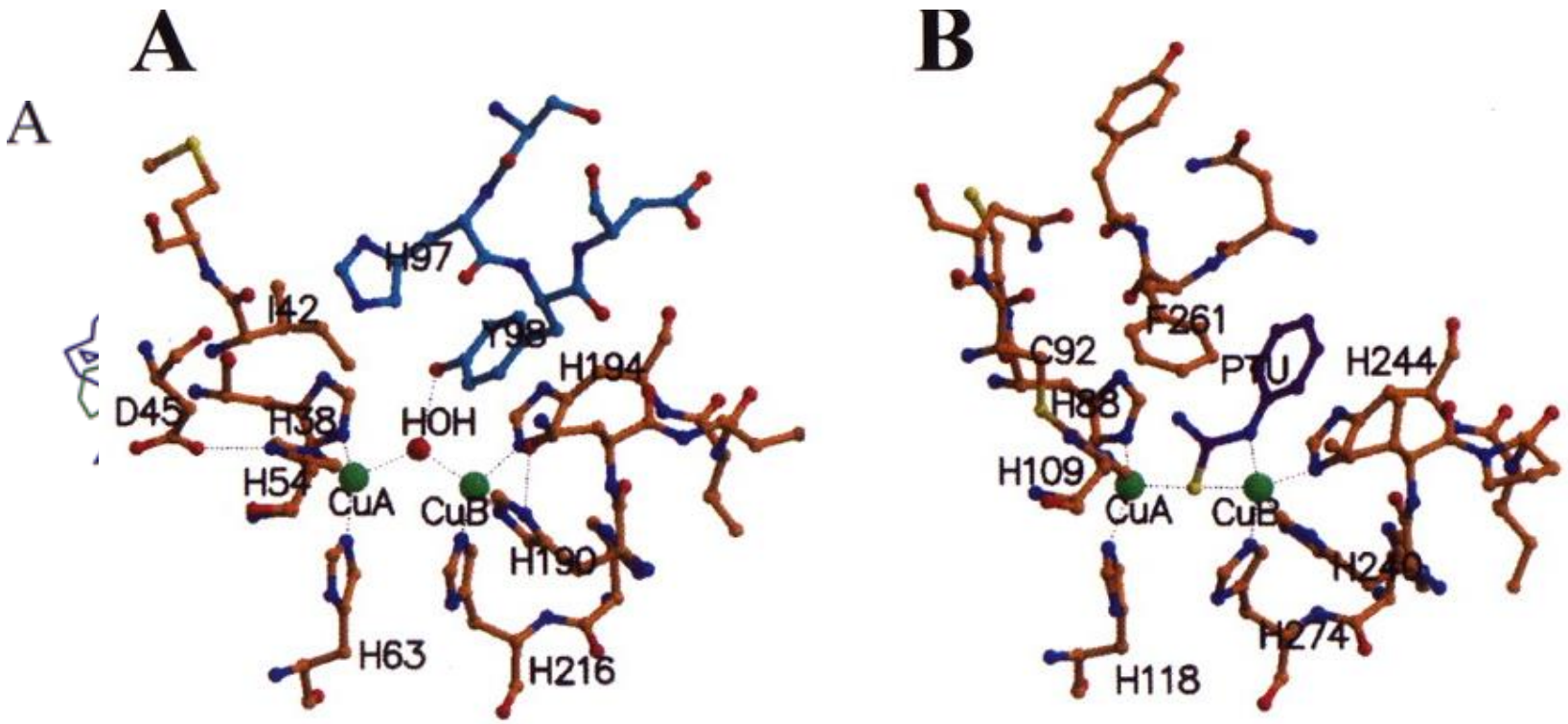


Tyrosinase structure

- All tyrosinases and catechol oxidases and hemocyanins have a binuclear type 3 copper center
- Each copper atom is coordinated with three histine residues
- Both a catechol oxidase from sweet potato and a tyrosinase from *Streptomyces castaneoglobisporus HUT 6202* have been crystallized and the structure solved
- Steptomyces structure has “caddie” protein
- Pymol

Tyrosinase, catechol oxidase, and octopus hemocyanin

seq catechol oxidase and tyrosinase (25%), tryosinase and hemocyanisn (-)



A tyrosinase (blue from caddie), **B** catechol oxidase (notice the phe F261) - PTU is inhibitor

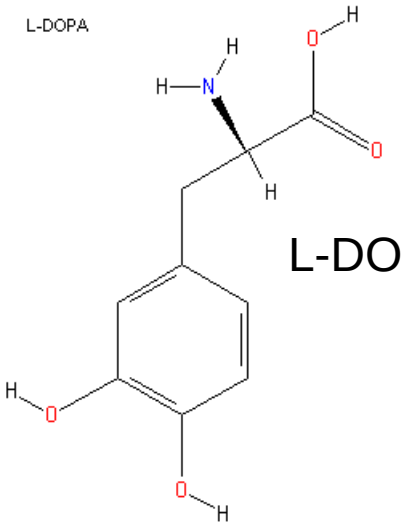
Matoba (2006)

(hemocyanin shield blocks active site)

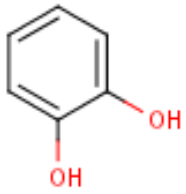
Mushroom Tyrosinase

- Different isoforms have differnet activities. None show significant tyrosine hydroxylation. Monophenol oxidase activity is lower (almost nonexistent) than diphenol oxidase activity

- Ex. Cap skin – highest to lowest. THIS IS A MIXTURE OF MANY ISOFORMS!
  - Catechol
  - 4-methylcatechol
  - L-dopa
  - Pyrogallol

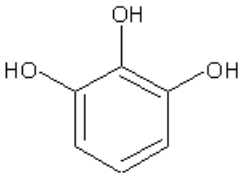


L-DOPA



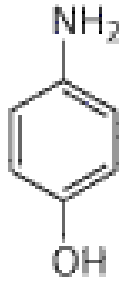
Catechol

Pyrogallol

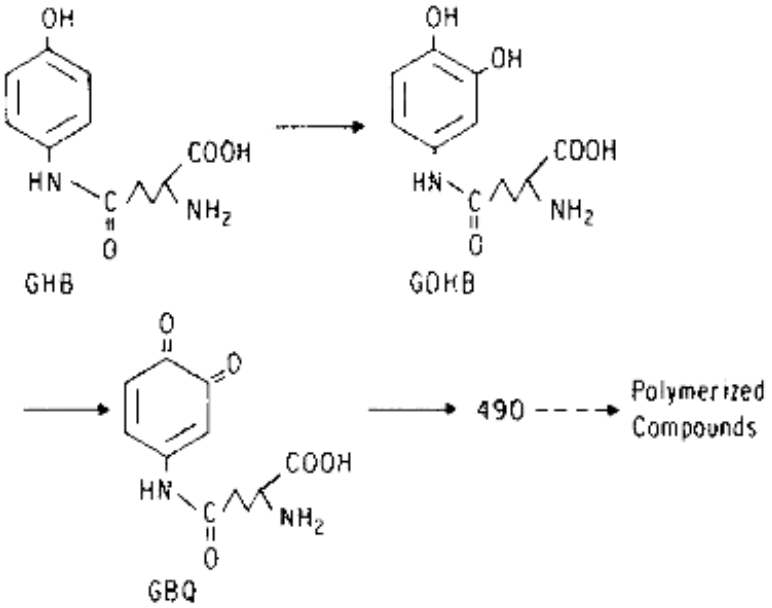


Mushroom tyrosinase: *putative* substrates *in vivo*

- p-aminophenol



- glutaminyl-4-hydroxybenzene (GHB)



Browning seems to be controlled by decompartmentalization

Mushroom Tyrosinase Sequence

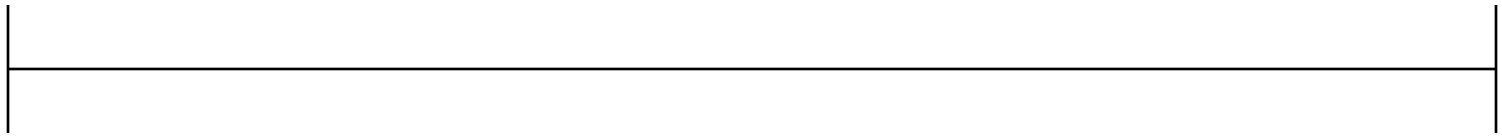
- Wichers et al. (2003) Appl. Microbiol. Biotechnol 61: 336-341
- Two genes AbPPO1 and AbPPO2 (*notice safe use of PPO*)
- Proteolysis
- Many glycosylation and phosphorylation sites
- Protein produced in *E.coli* is recognized by antibody but is NOT active
- 90% certainty no other PPO genes

Glycosylation

- 10 bands on IEF gel ?? (Flurkey)
- Low pI bands are due to glycosylation - review
- Quick review of glycosylation:
  - 2 types N-linked and O-linked
  - **N-linked**: N-acetylglucosamine is linked to the amide nitrogen of asparagine (asp-X-thr or asp-X-ser), more moieties are bound to the glucosamine (VARIABLE)
  - **O-linked**: moieties are linked to ser or thr, 1 - 1000 moieties VARIABLE
  - **MICROHETEROGENITY**
  - NO GENERALIZATIONS REGARDING FUNCTION
  - **Any takers?**

Final thought:

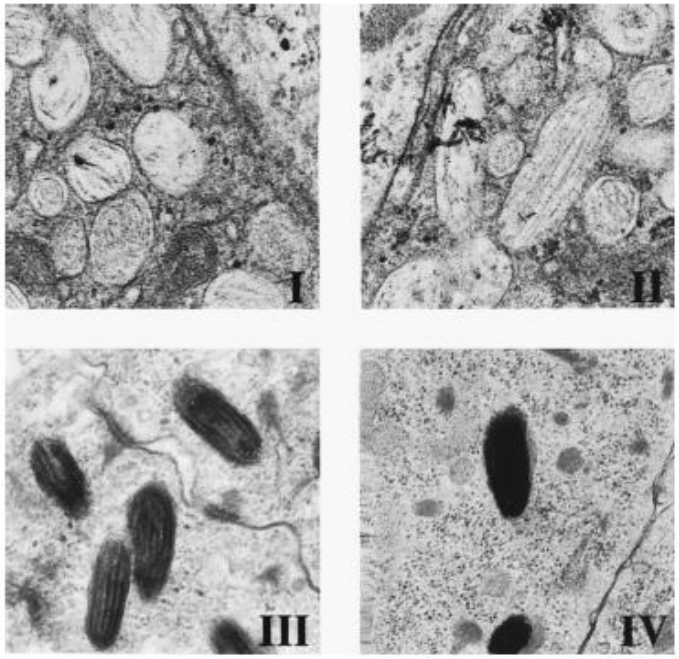
- Should we rename the lab “Characterization of catechol oxidase from mushroom” ?
- Tyrosinase vs. catechol oxidase
  - Black, white or gray?



tyrosinase ***Catechol oxidase***

Anybody want to try a site-directed mutagenesis approach?

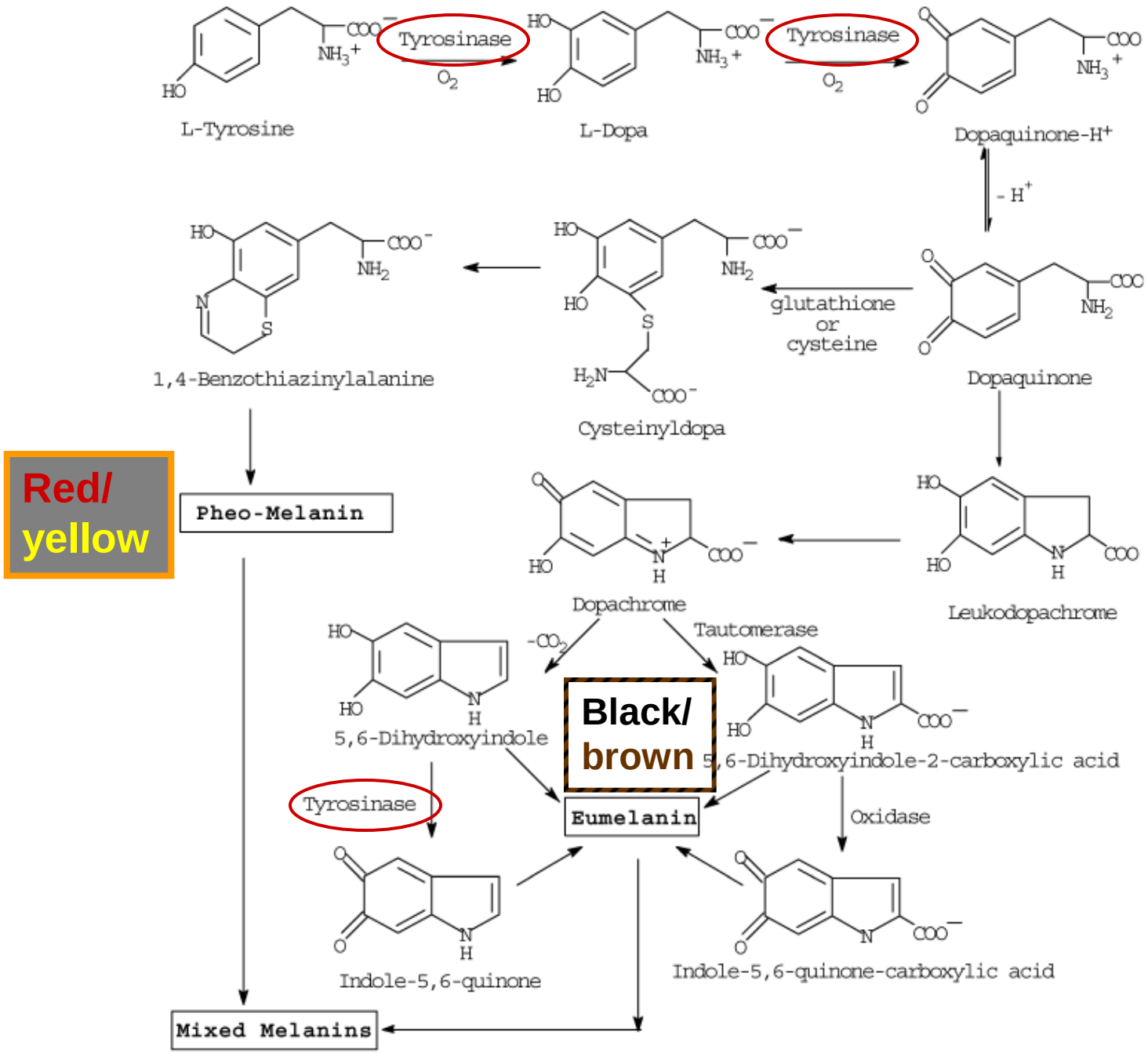
Human Tyrosinase



- Tyrosinase is a critical enzyme in melanin production
- It catalyzes the hydryoxlation of tyrosine and DOPA
- Hydroxylation of tyrosine is the rate-limiting reaction in melanin biosynthesis
- After DOPAquinone is formed melanin synthesis is spontaneous *in vitro*.
- All enymes are membrane bound

Melonocytes synthesize melanin in melanasomes and transport the mature melanosomes to keratinocytes.

Scheme 1. Pathway of Melanogenesis (*13, 16, 17*)



Human Tyrosinase Family

- The human tyrosinase gene family has three members: tyrosinase (TYR), tyrosinase related protein 1 (TRP1), and tyrosinase related protein 2 (DCT,TRP2).
- They bind different divalent metal cations and have different catalytic properties. They act in a complex. DCT1 (trp2) binds zinc, tyrosinase binds copper, and it is unclear which cation is bound by TRP1.
- Trp1 and tyrosinase share 40% AA identity. Dct and tyr share 32% identity; both were identified because they are recognized by tyrosianse antibodies.
- SEE NEXT SLIDE FOR TRP2 CONFUSION. MIGHT BE MORE IMPORTANT IN COMPLEX STABILIZATION.
- All three are involved in melanin biosynthesis.
- They do NOT have detectable levels of each others enzymatic activity.

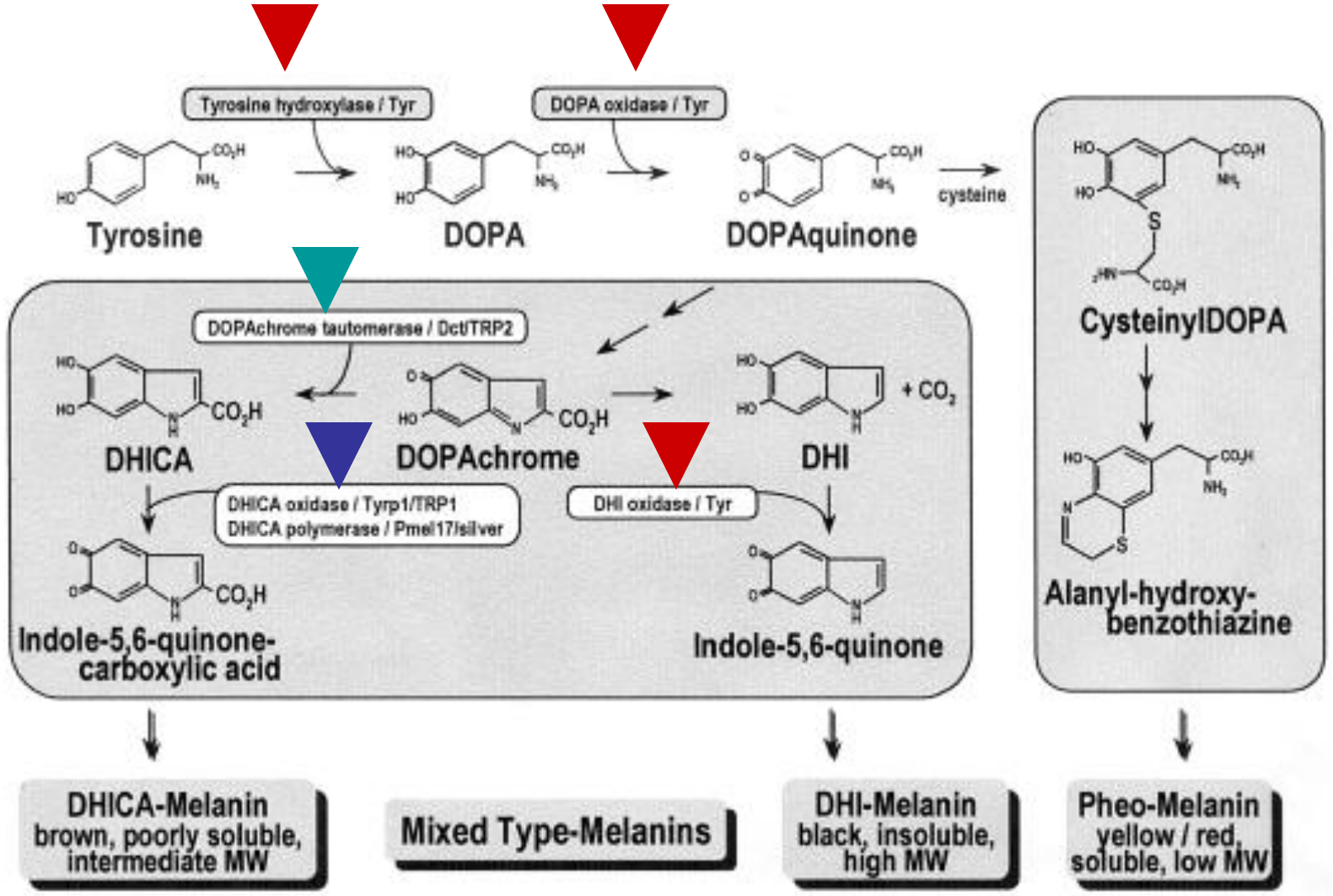
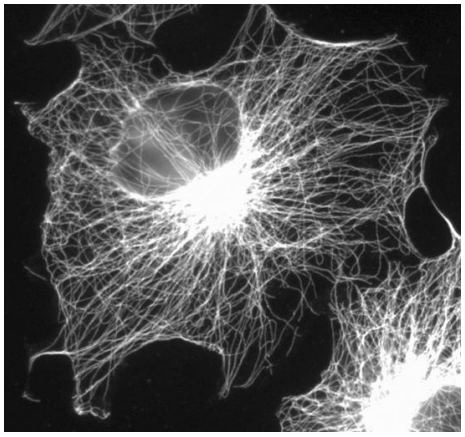


Fig. 220-4 Melanin biosynthetic pathway. The melanin pathway presents a summary of the known reactions and regulatory enzymes in eumelanogenesis and pheomelanogenesis.

Dopa is a required cofactor for tyrosinase hydroxylation.
Mouse tyrosinase can hydroxylate DHI but not DHICA, BUT human tyrosinase can use DHICA. Mouse Trp1 can use DHICA BUT human cannot! (99%)

Fishy Melanosome Movies (Borisy lab)

- Melanosomes are vesicles filled with melanin
- All the enzymes are in the membrane with the enzymatic subunit inside
- Melanosomes are moved along the microtubule tracks by kinesin (towards the outside) and dynien (towards the center)
- Caffeine inhibits dynein - MOVIE 1
- Melanosomes are moved along actin filaments by myosins; actin filaments
- Lantrunculin disrupts the actin network



Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)

EC 1.14.18.1

Accepted name: monophenol monooxygenase

Reaction: L-tyrosine + L-dopa + O_2 = L-dopa + dopaquinone + H_2O

Other name(s): tyrosinase; phenolase; monophenol oxidase; cresolase; catechol oxidase; polyphenolase; pyrocatechol oxidase; dopa oxidase; chlorogenic oxidase; catecholase; polyphenol oxidase; monophenolase; *o*-diphenol oxidase; chlorogenic acid oxidase; diphenol oxidase; *o*-diphenolase; tyrosine-dopa oxidase; *o*-diphenol:oxygen oxidoreductase; polyaromatic oxidase; monophenol monooxidase; *o*-diphenol oxidoreductase; monophenol dihydroxyphenylalanine:oxygen oxidoreductase; *N*-acetyl-6-hydroxytryptophan oxidase; monophenol, dihydroxy-L-phenylalanine oxygen oxidoreductase; *o*-diphenol:O2 oxidoreductase; phenol oxidase

Systematic name: monophenol,L-dopa:oxygen oxidoreductase

Comments: A group of copper proteins that also catalyse the reaction of EC 1.10.3.1 catechol oxidase, if only 1,2-benzenediols are available as substrate.

Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)

EC 1.10.3.1

Accepted name: catechol oxidase

Reaction: **2** catechol + O2 = **2** 1,2-benzoquinone + **2** H2O

Other name(s): diphenol oxidase; *o*-diphenolase; phenolase; polyphenol oxidase; tyrosinase; pyrocatechol oxidase; Dopa oxidase; catecholase; *o*-diphenol:oxygen oxidoreductase; *o*-diphenol oxidoreductase

Systematic name: 1,2-benzenediol:oxygen oxidoreductase

Comments: A group of copper proteins that act also on a variety of substituted catechols, and many of which also catalyse the reaction listed under EC 1.14.18.1 monophenol monooxygenase; this is especially true for the classical tyrosinase.

References

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Read about mechanism & biological function!