
Welcome to Japan! Welcome to Tokyo!
Recepción a Japón !!
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The Novel Hair Dyeing Technology by Using Melanin Precursor Prepared by an Aspergillus Tyrosinase

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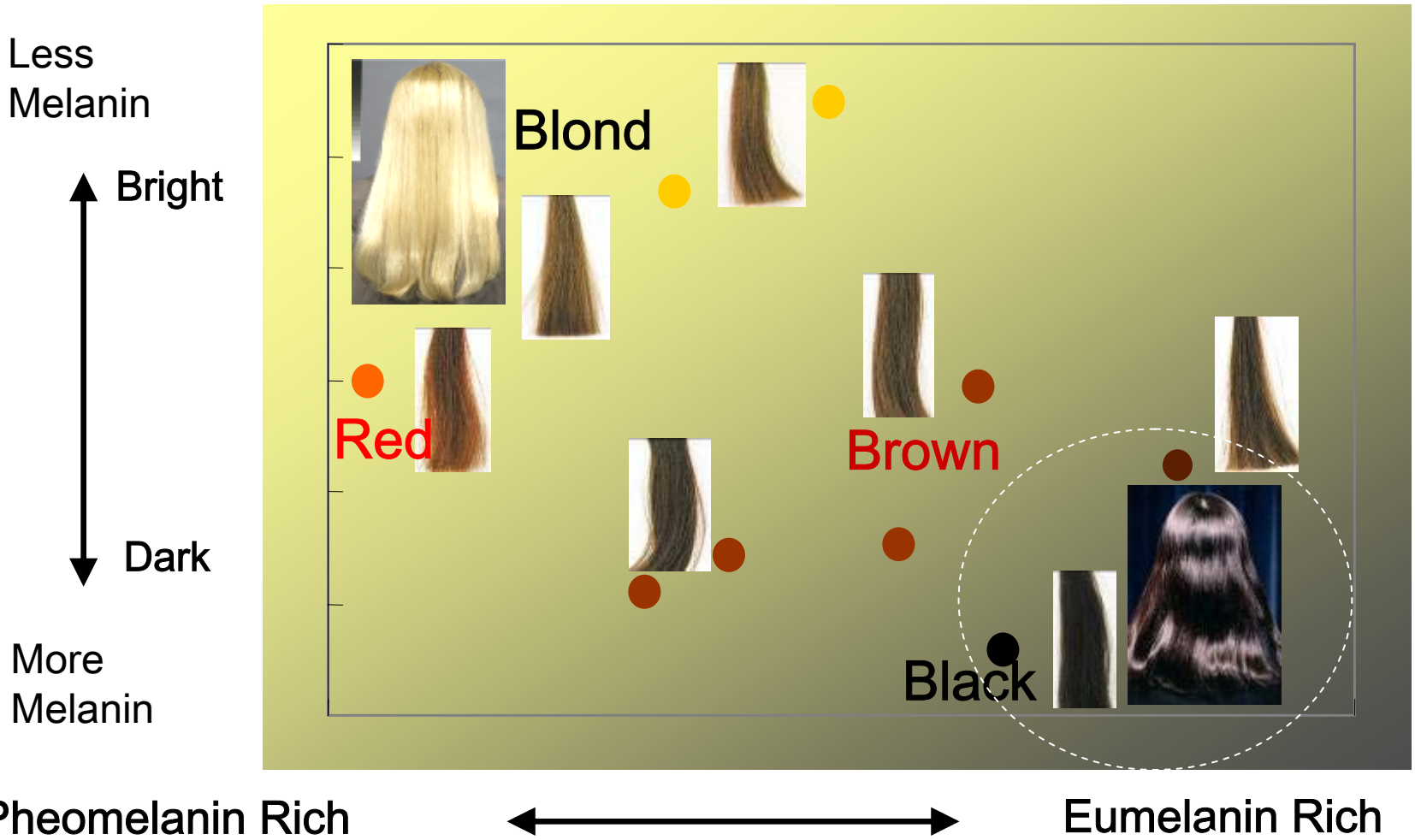
Traditional Japanese Sake Brewers

1. Background of Our Hair Research

Hair Research encountered Biotechnology in 2001.

Relationship between melanin and color of the hair

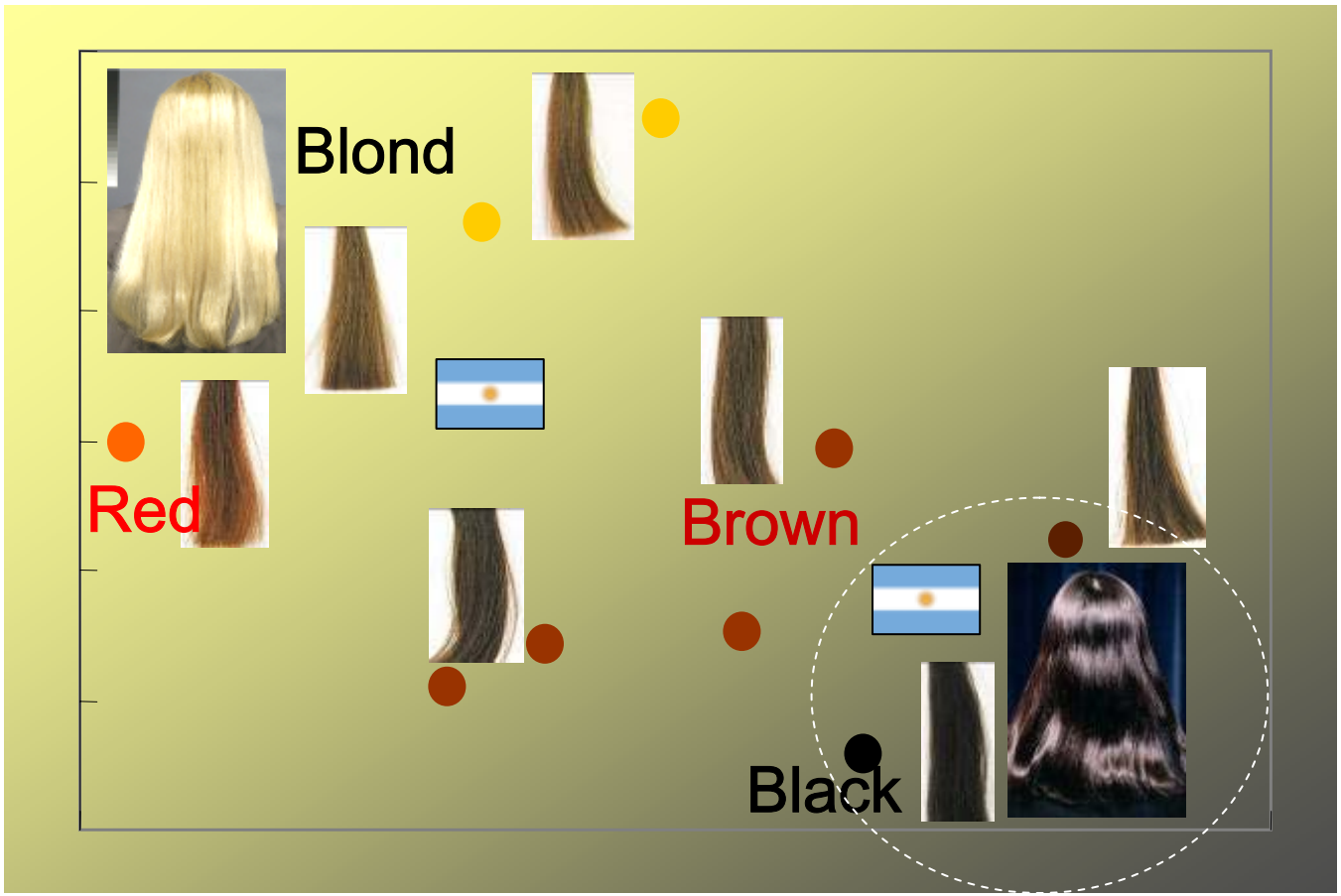
- Melanin type and amounts affect color and brightness of the hair -



Asian hair has more Eumelanin

Relationship between melanin and color of the hair

- Melanin type and amounts affect color and brightness of the hair -



Asian hair has more Eumelanin

Relationship between melanin and color of the hair

- Melanin type and amounts affect color and brightness of the hair -

Asian hair has more Eumela...

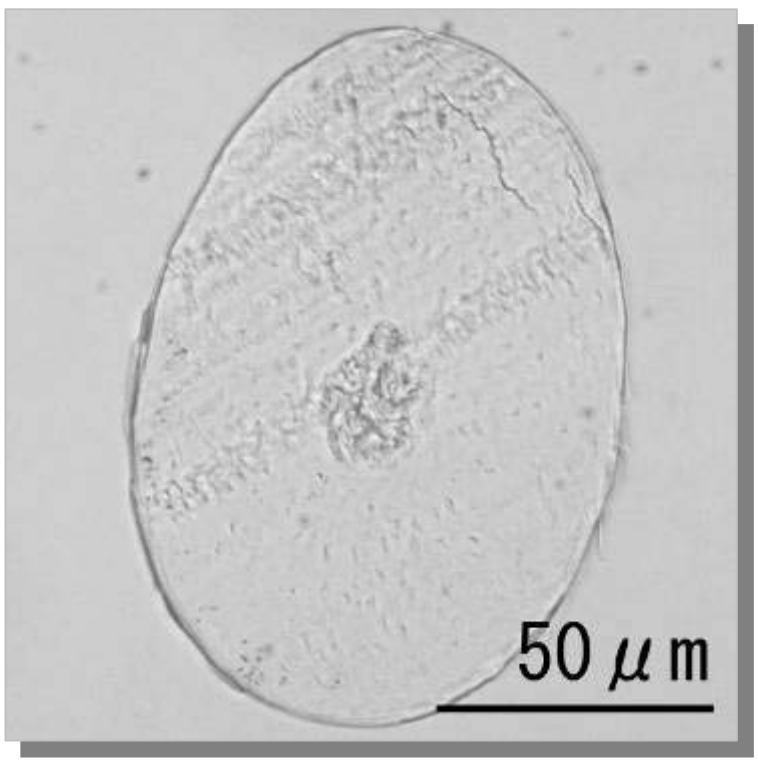
The difference between Gray and Black Hair

- The difference is only melanin -

Male hair sample from a Japanese (50's)

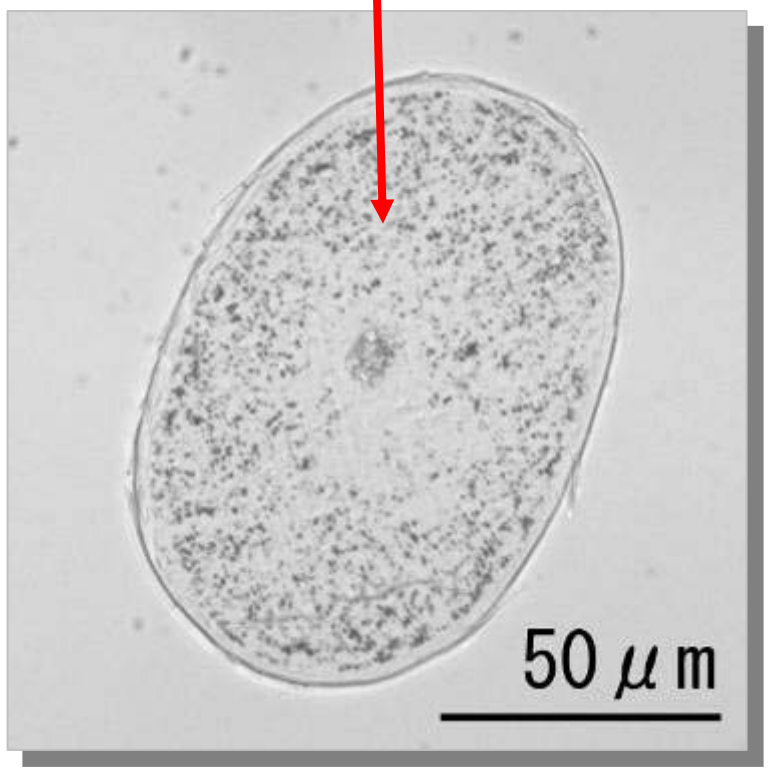
Gray hair

No melanin



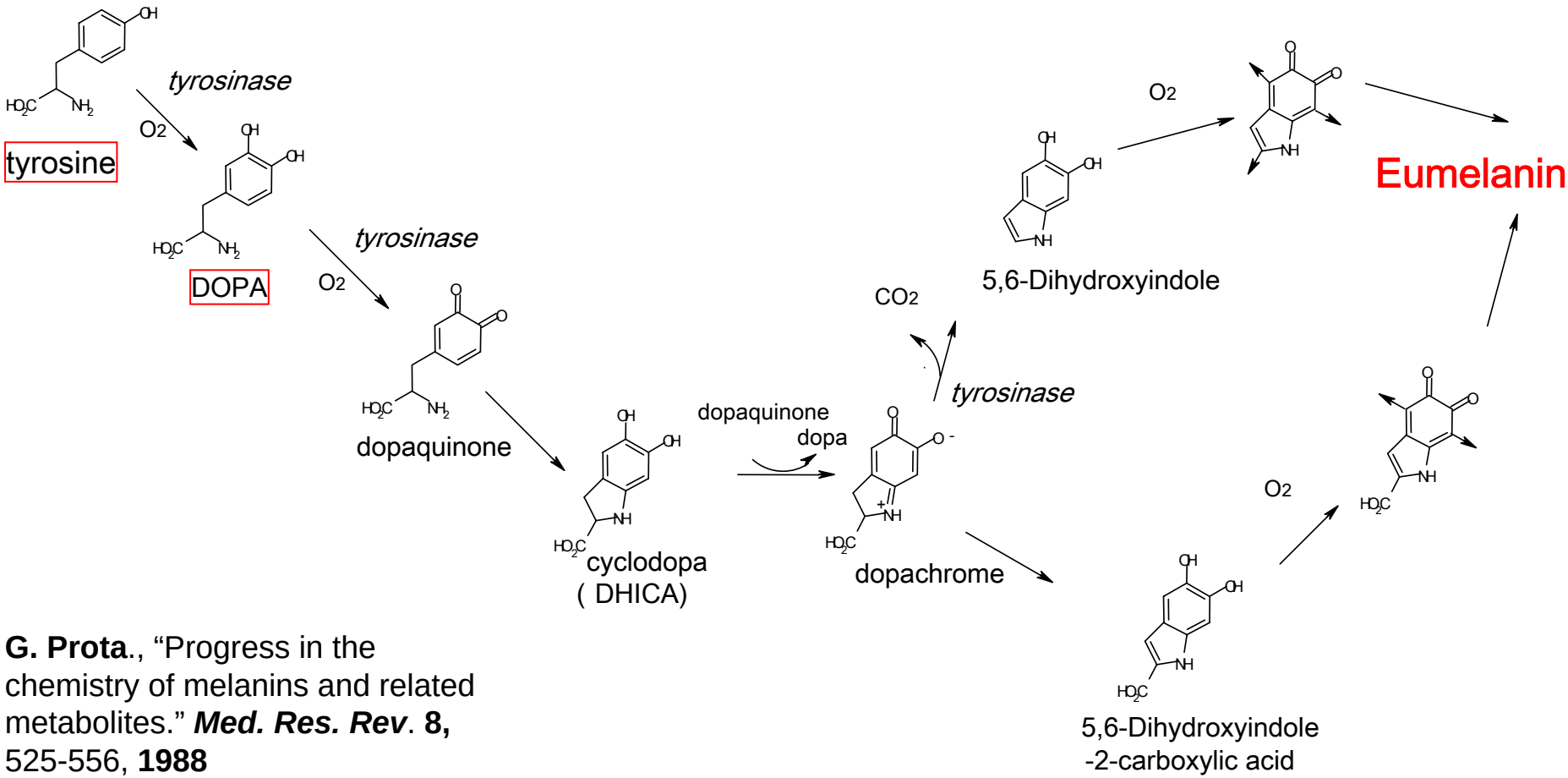
Black hair

Melanin Granules



Melanogenesis Eumelanin biosynthesis

- Eumelanin is a polymer made from Tyrosine or DOPA.
- This pathway is initiated by Tyrosinase oxidation reaction.
- Melanin precursors are converted to Melanin by Oxygen.



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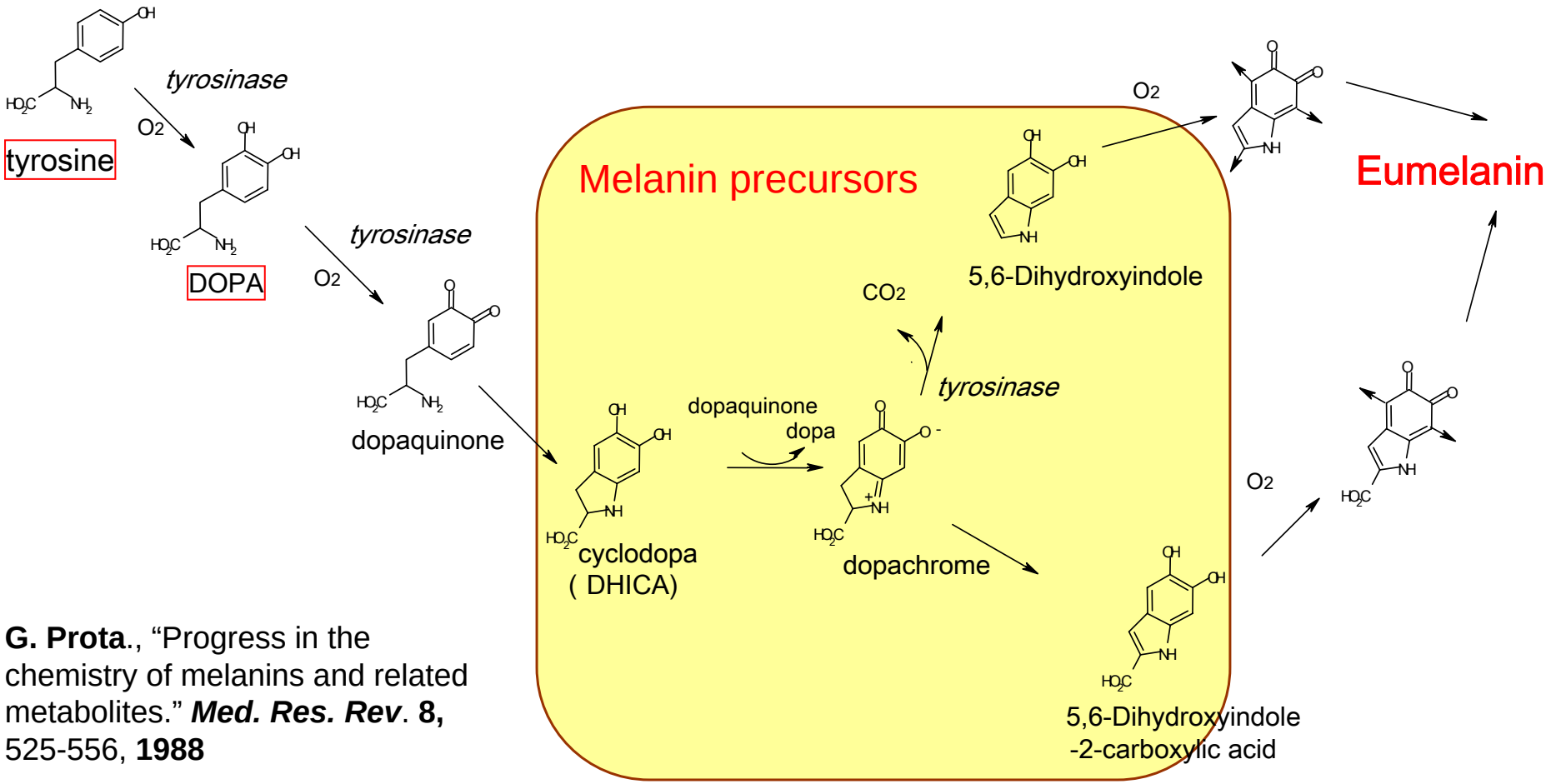

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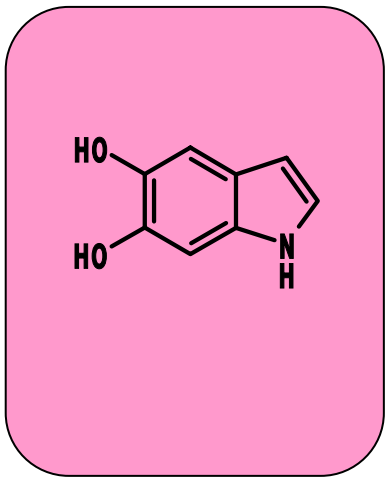
A new idea for hair dyeing system with melanin (precursor)

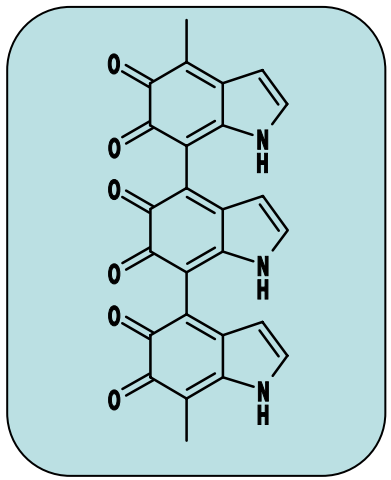
Melanin precursor



Into Hair (Cuticle)

O_2 (Air)

Melanin



Benefits

- Melanin and its precursors are **biological substances**.
- The Melanin precursor is small enough to penetrate hair.
- It converts to melanin by exposure to air.
- Melanin has a **natural** dark color.

Issues: The Melanin precursor is very unstable.
None of the natural compounds were industrially available.

By the way, we have traditional **biotechnology** in Japan.

Fungus and **yeast** have been used
in food manufacturing and various fermentation processes in Japan for over 1000 years.

Hair Research encountered Biotechnology in 2001.

02. Preparation of Melanin Precursor using fungus Tyrosinase

Manufacturing new ingredient from natural resources using same process as in nature.

Collaboration between Kao & Gekkeikan

Japanese Sake manufacturing

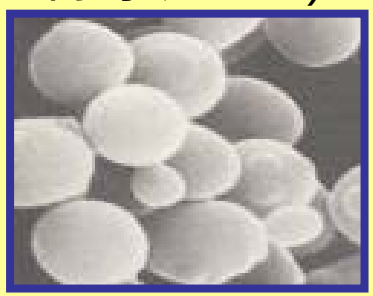
GEKKEIKAN
For Your Lifestyle Taste

Japanese Traditional Biotechnology

Rice

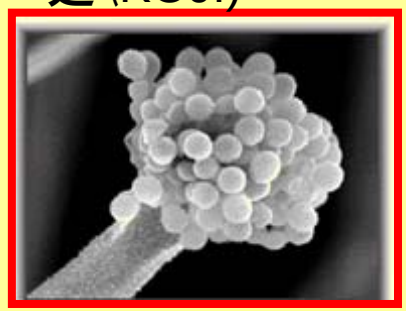
Clean Water

酵母 (KOBO)



Saccharomyces cerevisiae (yeast)

麹 (KOJI)



Aspergillus oryzae fungas)

酒造り (SAKE Dukuri)
Alcohol Fermentation

日本酒
Japanese SAKE

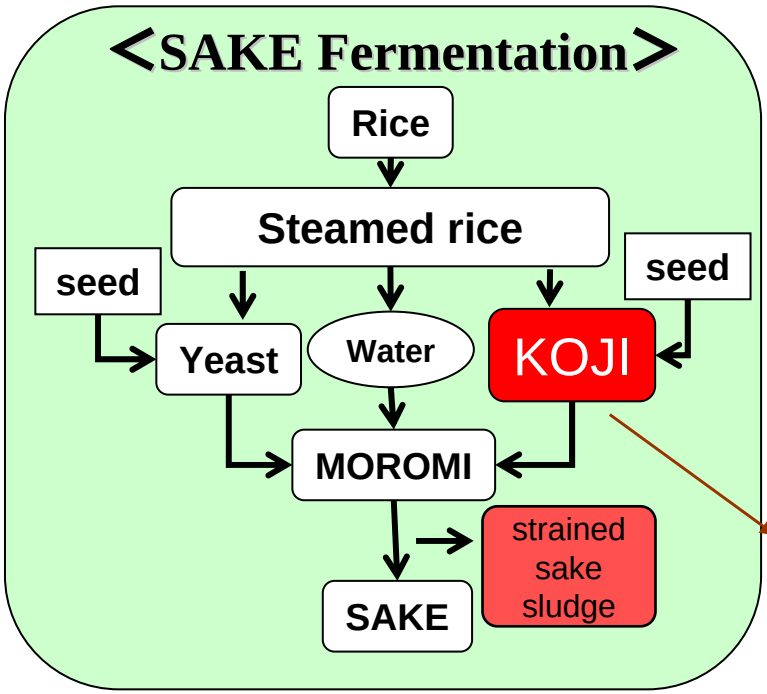
Traditional Brand from 17th century (400 yrs)

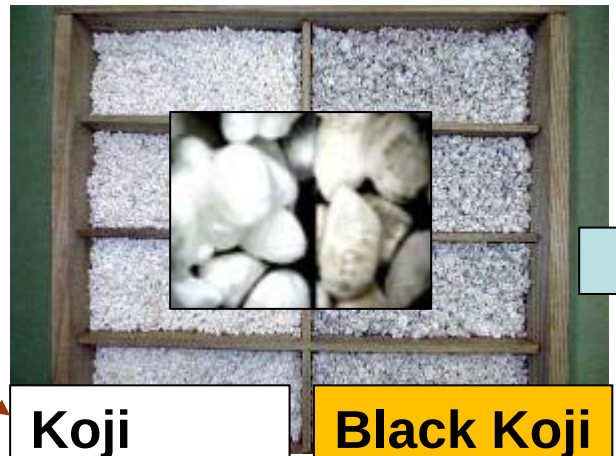
Koji (Normal)

Black Koji (Irregular)

There was a color problem, occasionally, during 1950s and1960s.

Fungal melanin formation in the process of fermentation.





Koji (Normal)

Black Koji (Irregular)

Melanin formation

As a result, the Black Koji was identified,
and the Melanin formation mechanism was found.

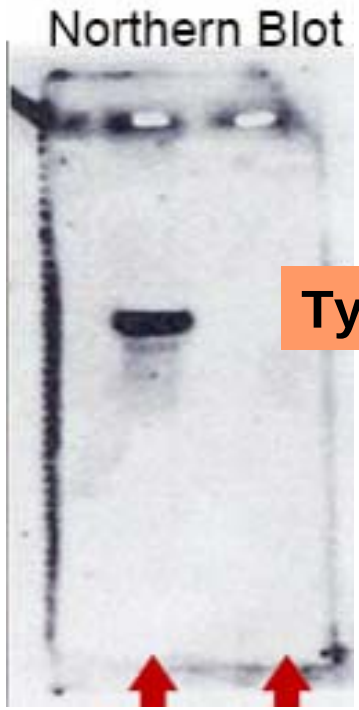
Tyrosinase from *Aspergillus oryzae* makes melanin.

1.Obata *et al*, “Cloning of a Novel Tyrosinase-Encoding Gene (melB) from *Aspergillus oryzae* and Its Over expression in Solid-State Culture (Rice Koji)” ***J. Biosci. Bioeng***. **97** 400-405 2004.

Tyrosinase was found to be expressed in Black Koji.

Black Koji

Koji : Fermented rice with *Aspergillus oryzae*



Solid Surface Culture

Liquid Culture

Tyrosinase (enzyme)

The Japanese Sake company found a tyrosinase strongly expressed in Black Koji.

New Collaboration research was started

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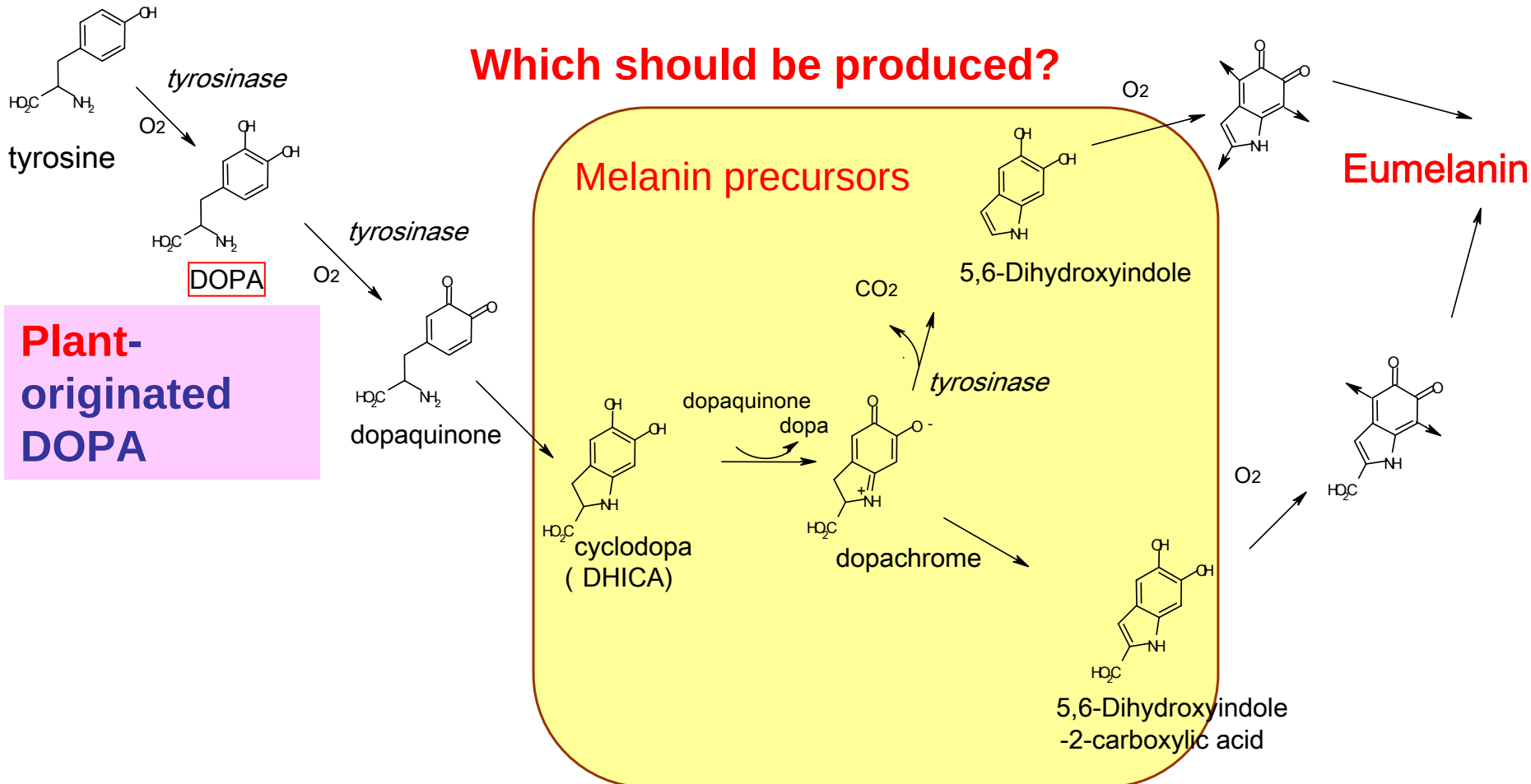
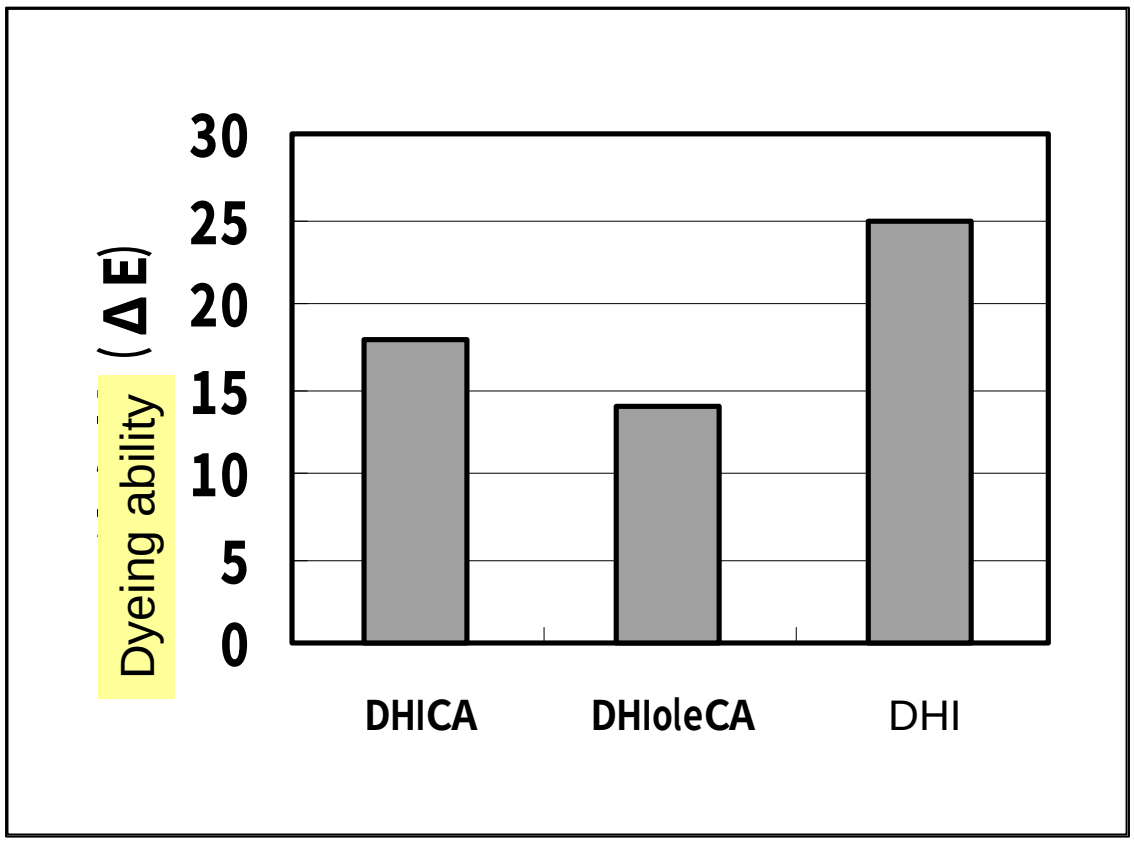
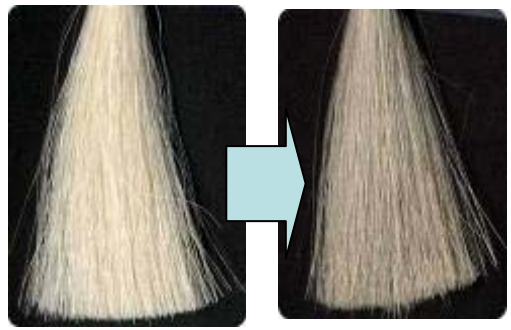


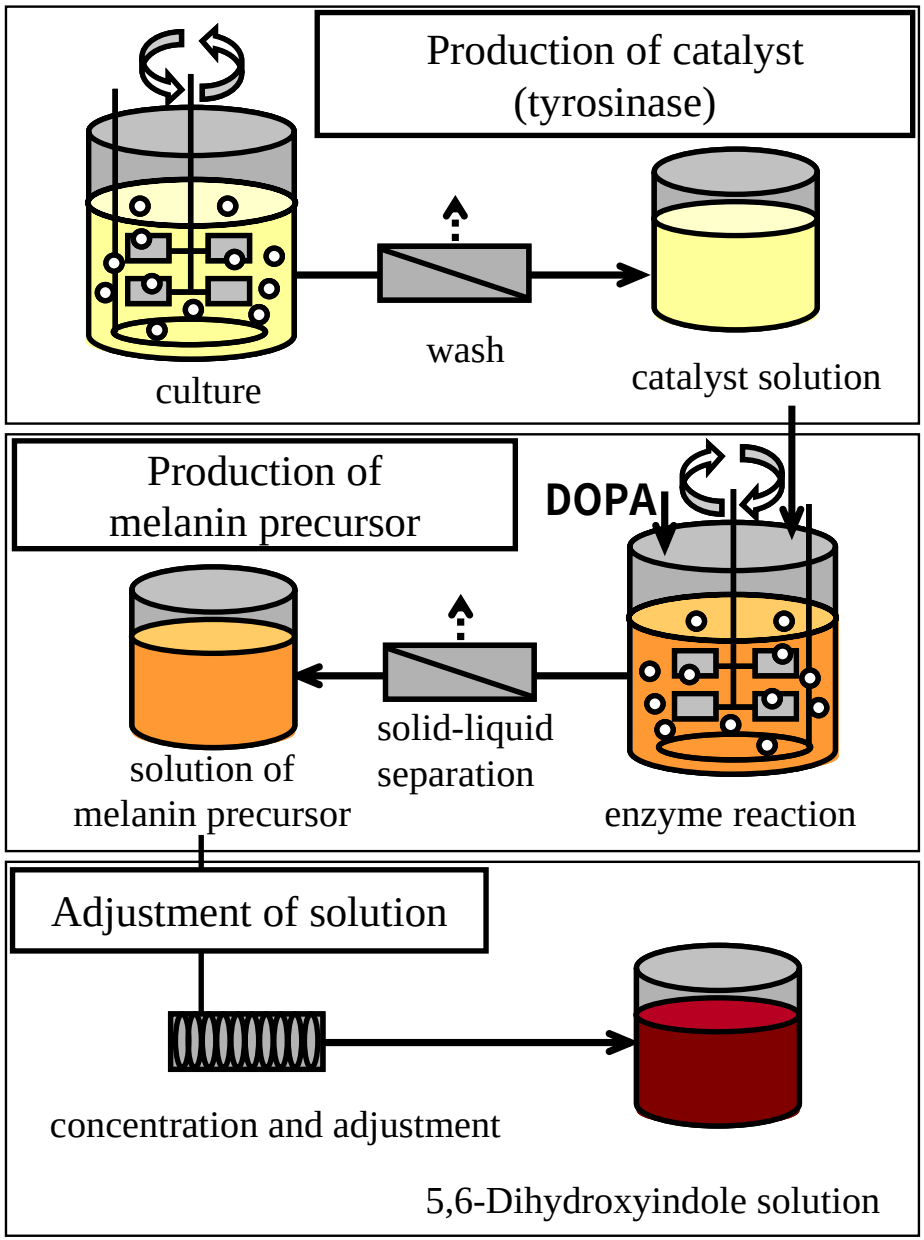
The dyeing ability of melanin precursors





DHICA : 5,6-Dihydroxyindline 2-carboxylic acid
DHIoleCA : 5,6-Dihydroxyindole 2-carboxylic acid
DHI : 5,6-Dihydroxyindole (DHI)
Dyeing condition : 3%Ammonia, 0.1% each compound, 3 times

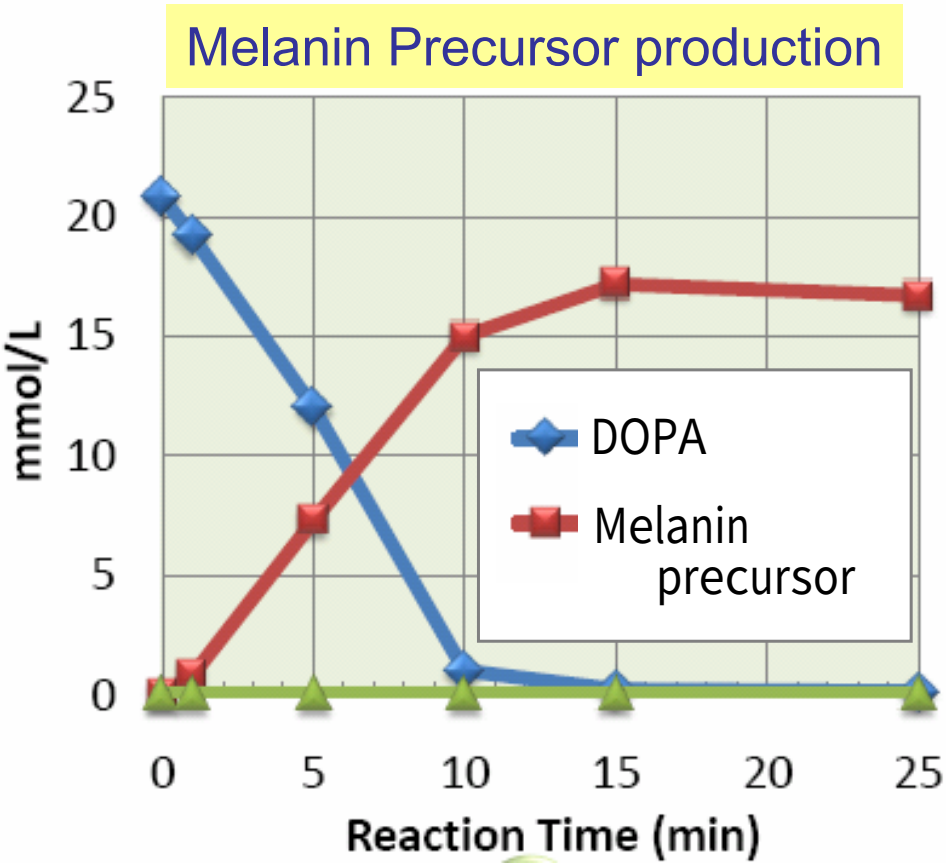
The total system for melanin precursor production

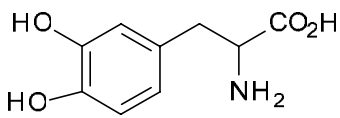


- Tyrosinase is produced by fermentation.
- Dopa is converted to Dihydroxyindole in tyrosinase process.
- The product is adjusted for cosmetics materials.

Melanin precursor production by fungus Tyrosinase

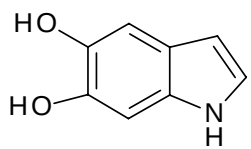
Melanin precursor was produced from Plant-originated Dopa.





DOPA (Plant-origin)

Melanin precursor (DHI)



- DOPA (the starting material) is of Plant-origin.
- Melanin precursor was produced by using the same process as in nature.
- The Melanin precursor is a sustainable compound.

03. Application of Melanin Precursor to Hair Dye

Development of a natural coloring product for gray hair.

New Concept of Natural Hair Dye

- The new dyestuff, 5,6-dihydroxyindole,has been manufactured from natural resources using a similar process to nature.
- It ensures "**True Natural Hair Color**" by recovering color by melanin.
- Air oxidation system without H_2O_2
  - Gentle to hair: less damage
  - Easy to use: no mixing, no long waiting.

Formulation of the Hair Dye

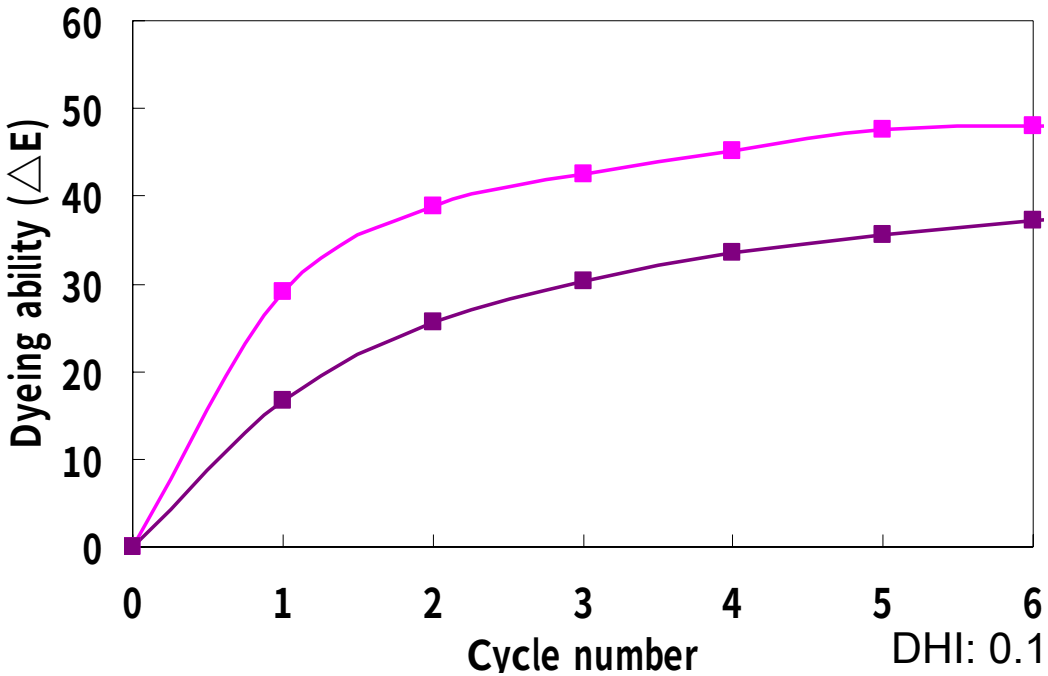
Foam type: Ease of Use

Aerosol: maintain stability by Oxygen shutoff

How to Use: 20g of the product was applied to hair. After standing for 5 min. hair is washed with shampoo.

The relationship between DHI conc. and Dyeing ability

Dyeing image

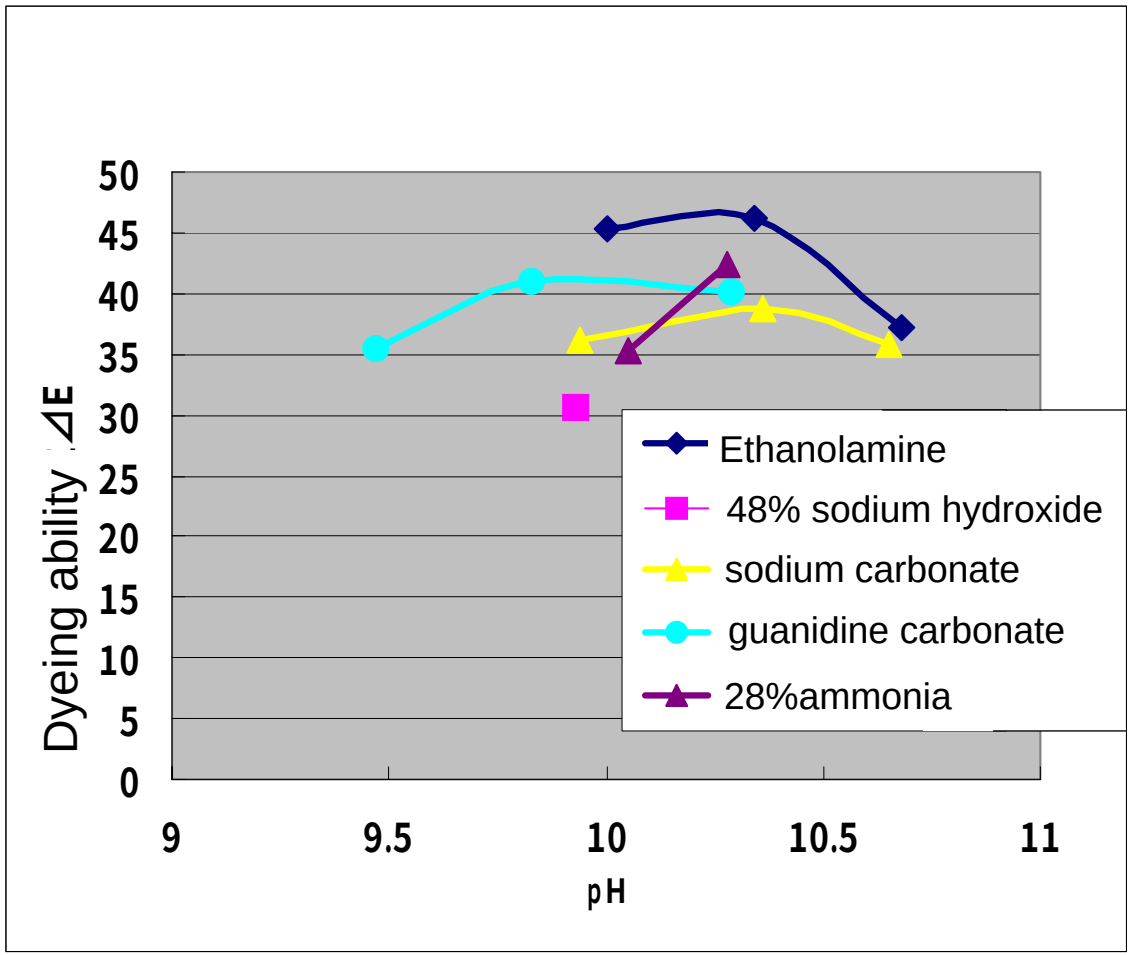


DHI: 0.1% or 0.3% + 3% ammonia
Procedure: Chinese grey hair, RT, 5min, shampooed and dried,
This treatment was repeated as indicated.

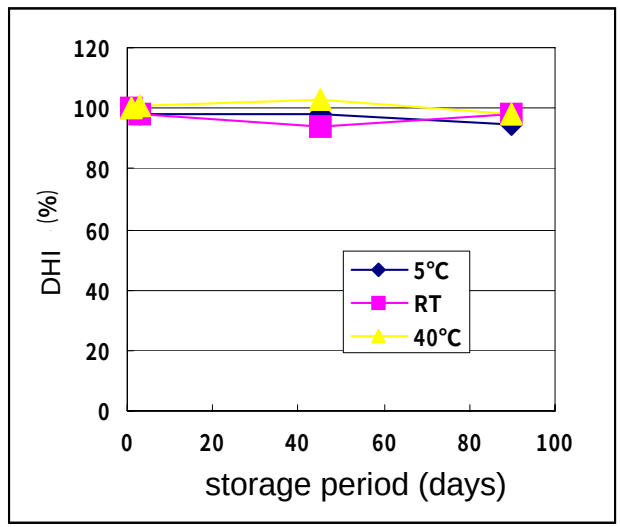
DHI at 0.1 - 0.3% dyed gray hair gradually.

The effect of alkaline and pH in Dyeing ability

pH 10 with ethanolamine was most effective



Melanin precursor in the formulation was stable for 6 months



Brown and Ash shades were achieved.

Brown shade

Ash shade

DHI: 0.3% + 1% Ethanolamine + Antioxidants
Procedure: Chinese grey hair, RT, 5min, shampooed and dried,
This treatment was repeated up to 5 times.

Melanin precursor is converted to Melanin by air

Dyeing ability: + +

Dyeing ability: ±

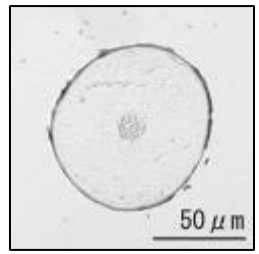
Melanin formation by air

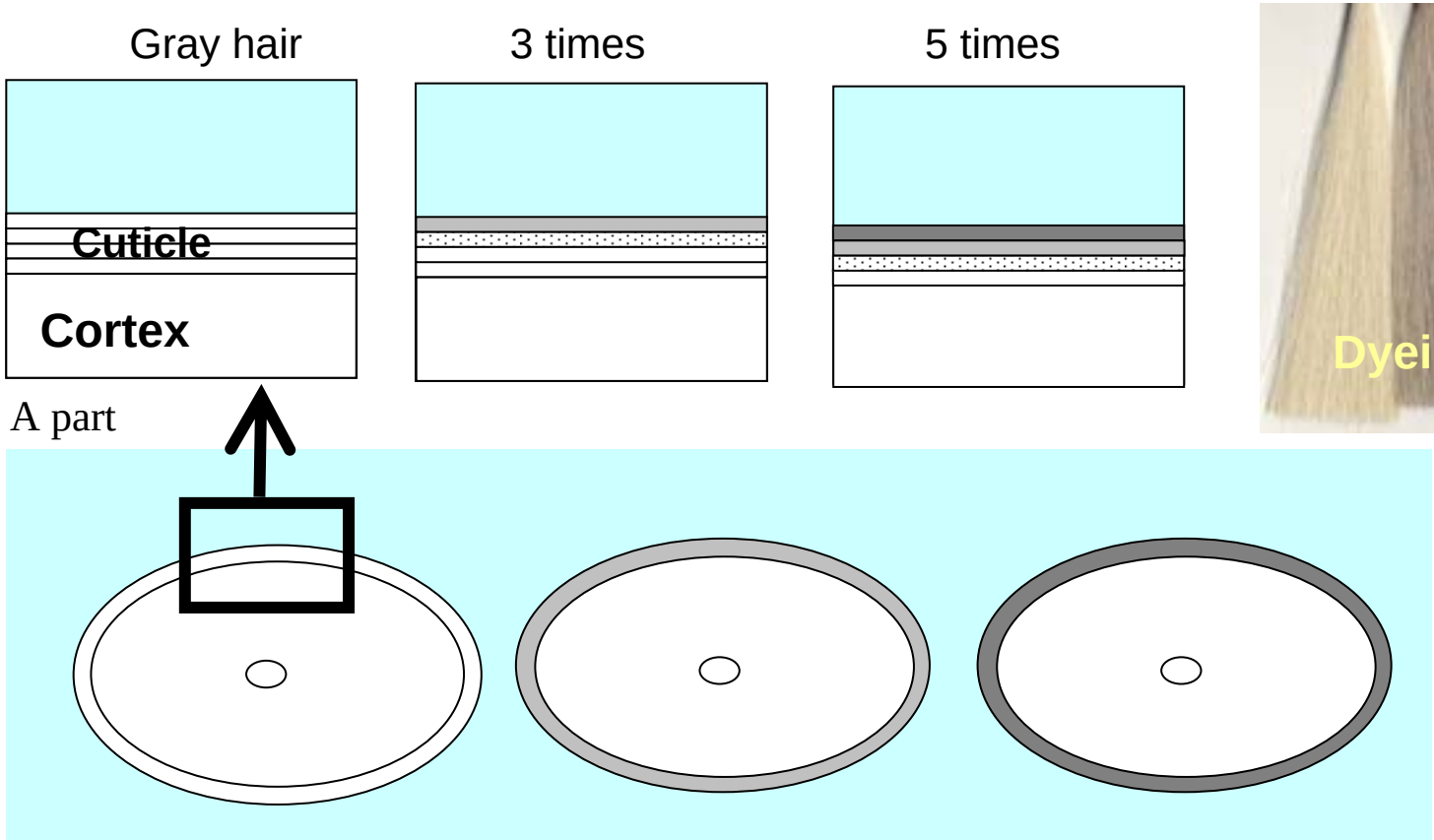
Easy to use: no mixing, no long waiting period

The mechanism for dyeing with melanin precursor

1. Melanin precursor can penetrate into the cuticle of hair.
2. Melanin is formed by exposure to air.
3. Gray hair is gradually dyed by step-wise use (3-5 times).
4. The final color of hair then looks more natural.

Cross section





How to Use

- Treatment
- **5 min.**
- Shampoo

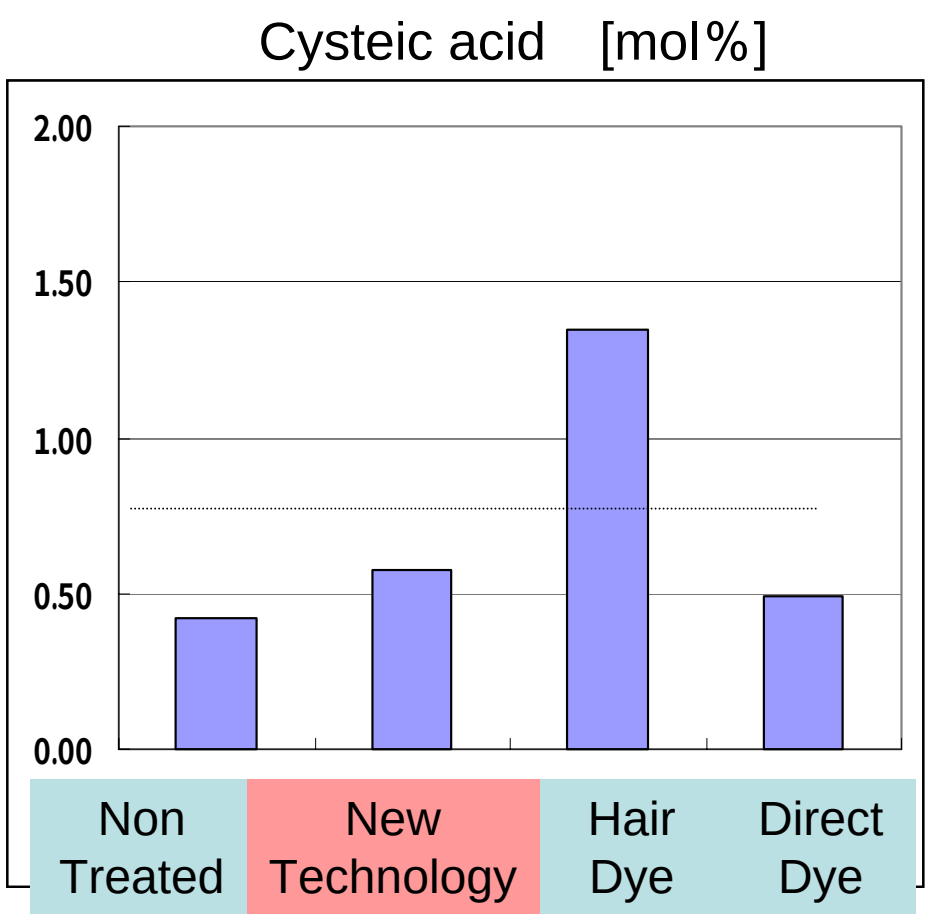
Dyeing ability

Panelist results

- Each repeated treatment brought natural change.
- The color of hair became very natural.
- The color only faded after application was stopped.

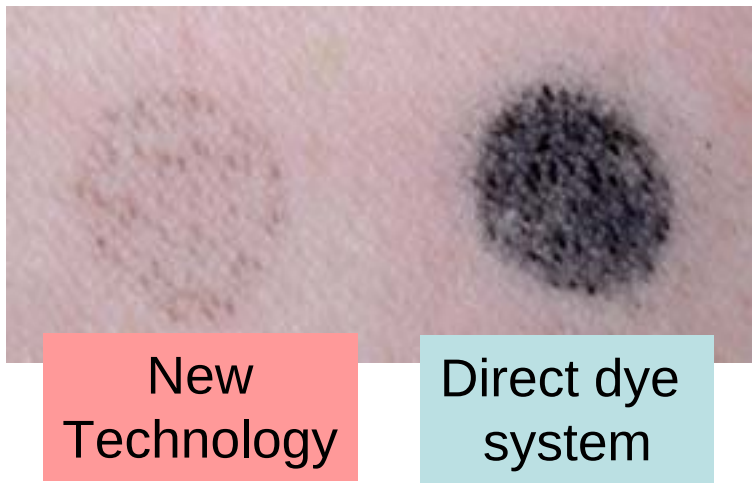
Advantages of the new technology

Hair damage level



Hair → Hydrolysis → A.A. analysis (HPLC)

Skin staining



Skin → 30 min. → Washing with water

A New Natural Hair Dye

- The new dyestuff from natural resources
  - DHI has been manufactured using a similar process to nature.
- Restoring Melanin into gray hair.
  - Recovery of natural color by melanin
- Gentle to hair and Easy to use
  - Air oxidation system without H_2O_2

Thank you for your attention!

Gracias mucho!!

Gracias mucho!!
Argentina!