

# AMPK activation: its effects on melanogenesis

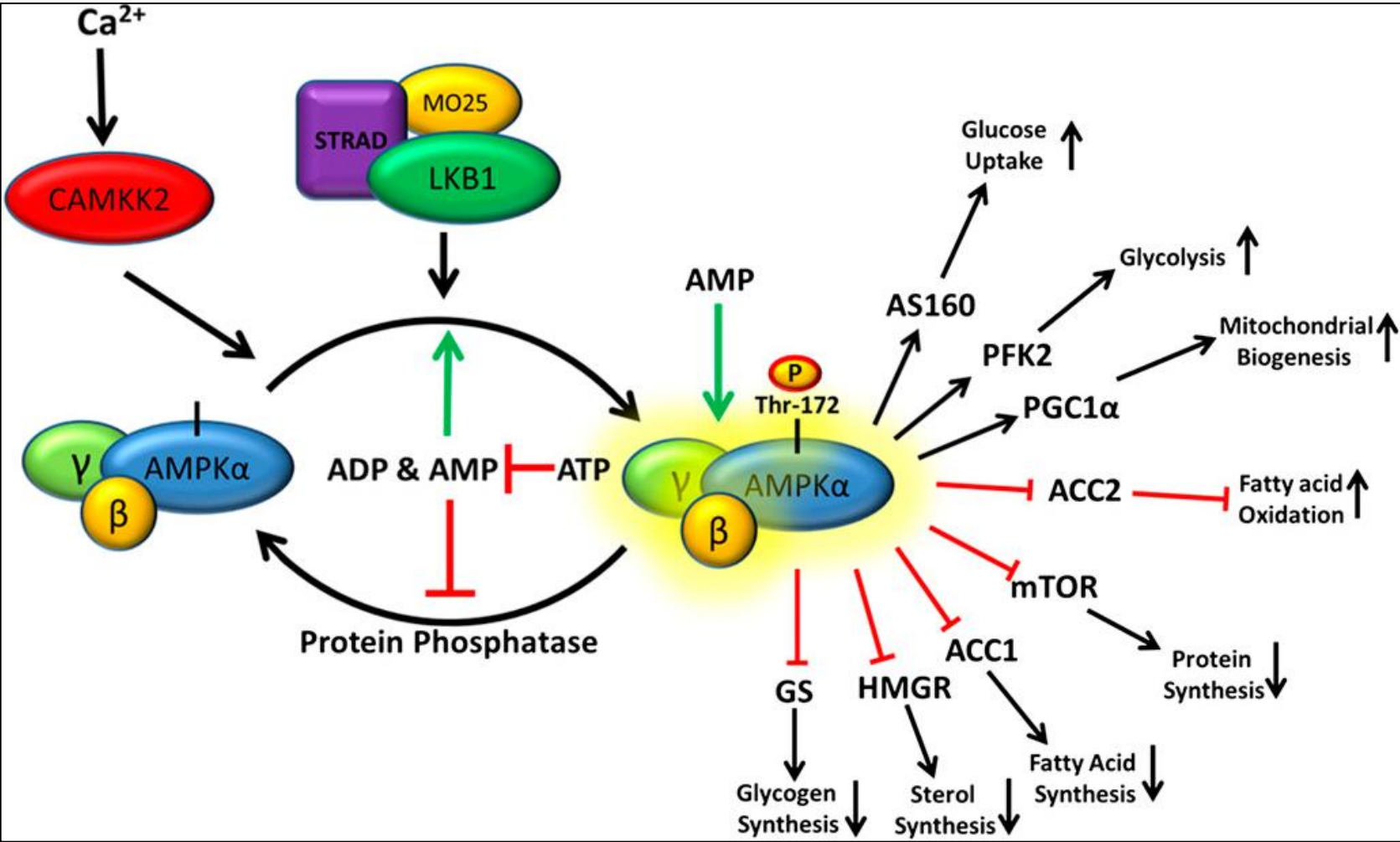
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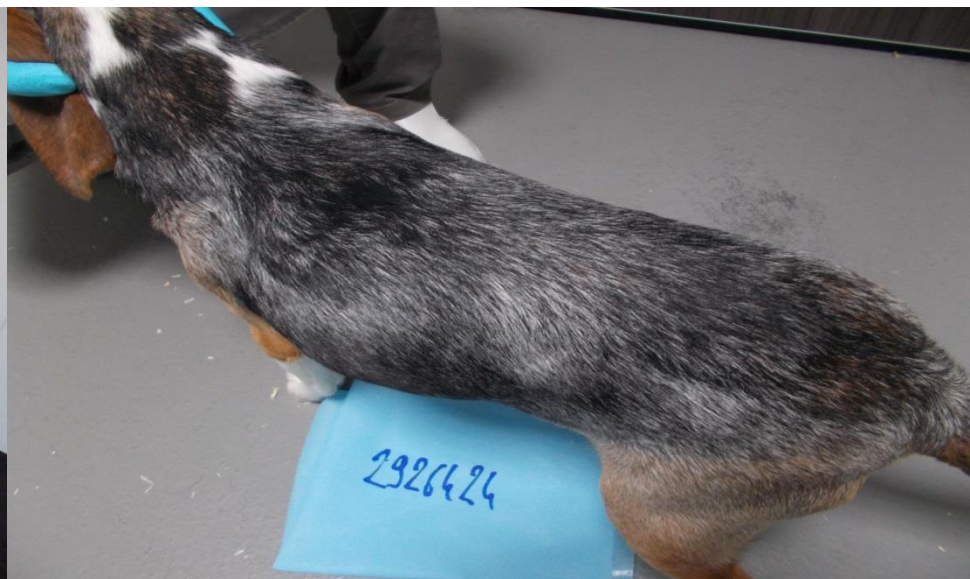
# AMPK activation: Effects



# AMPK activation: 14-day oral dosing in conscious telemetered dogs - Coat alterations



Untreated  
dog(same age)



Dog N° 2918537

# AMPK activation: Study design

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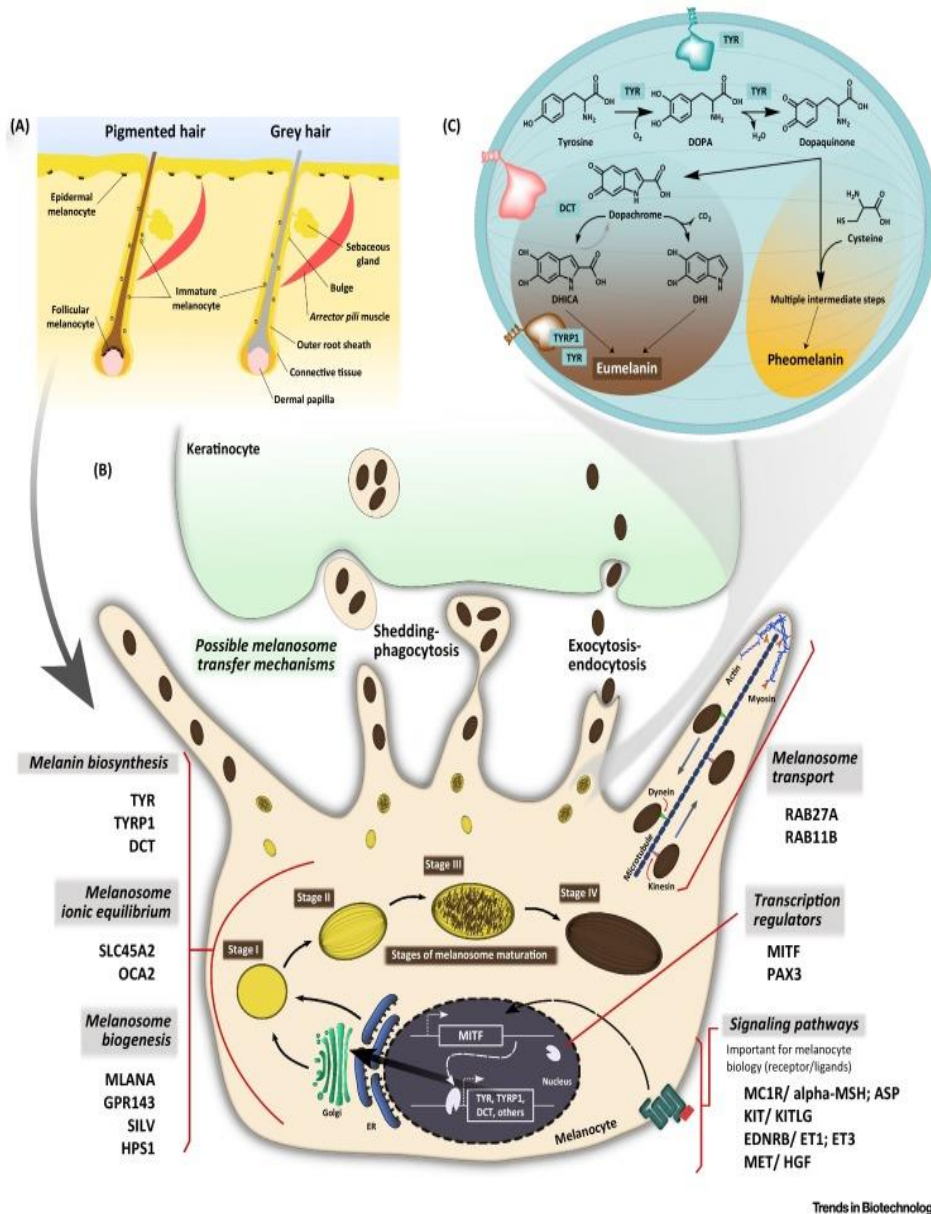
- **Animals** 1M/1F
- **Dose** 5 mg/kg
- **Duration of treatment** 4 weeks
- **Recovery period** ~ 6 weeks
- **Investigations**
  - Clinical observations: Daily
  - Detailed fur observations:  
Pre-treatment, at the end of Weeks 2, 4, 8 and 10
  - Histopathology of dermal biopsy samples:  
Pre-treatment, at the end of Weeks 2, 4, 8 and 10
  - Toxicogenomics of dermal biopsy samples :  
Pre-treatment, at the end of Weeks 2 and 4
  - Clinical Pathology: Pre-treatment, Days 5, 28 and 70
  - Ophthalmology: Pre-treatment, at the end of Weeks 4 and 10
  - Toxicokinetics: Days 1 and 28

# AMPK activation: In life results

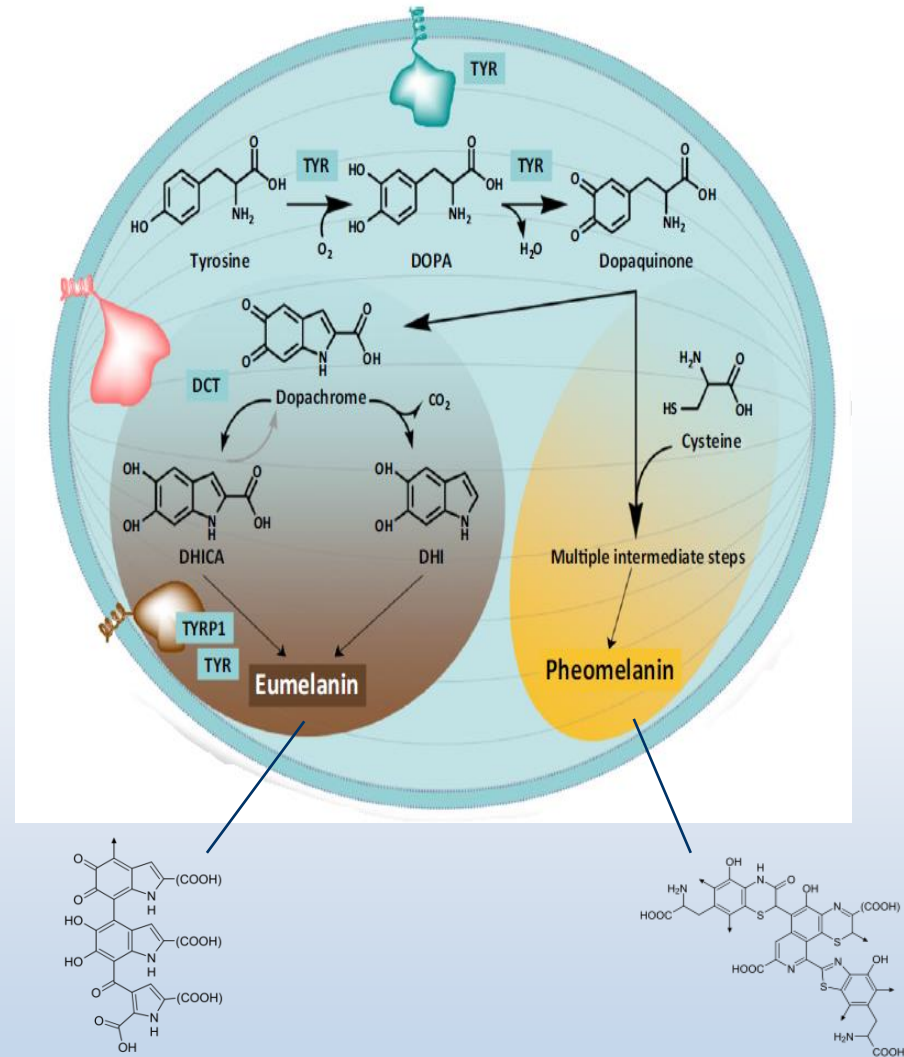
- Clinical observations:
  - No toxicologically relevant clinical signs were observed.
- Detailed fur observations:
  - No toxicologically relevant signs after 4-weeks
- Clinical pathology: Pre-treatment, Days 5, 28 and 70.
  - No toxicologically relevant clinical signs were observed.
- Ophthalmology:
  - No test item-related ocular changes were noted
- Toxicokinetics: Days 1 and 28

Day	Animal (sex)	C(max) [nmol/L]	AUC(0-24h) [nmol·h/L]	t(max) [h]
1	No. 201 (m)	1360	5770	1
	No. 251 (f)	2810	10400	1
28	No. 201 (m)	1020	5130	2
	No. 251 (f)	3560	19200	2

# AMPK activation: Melanocyte function in hair follicle pigmentation

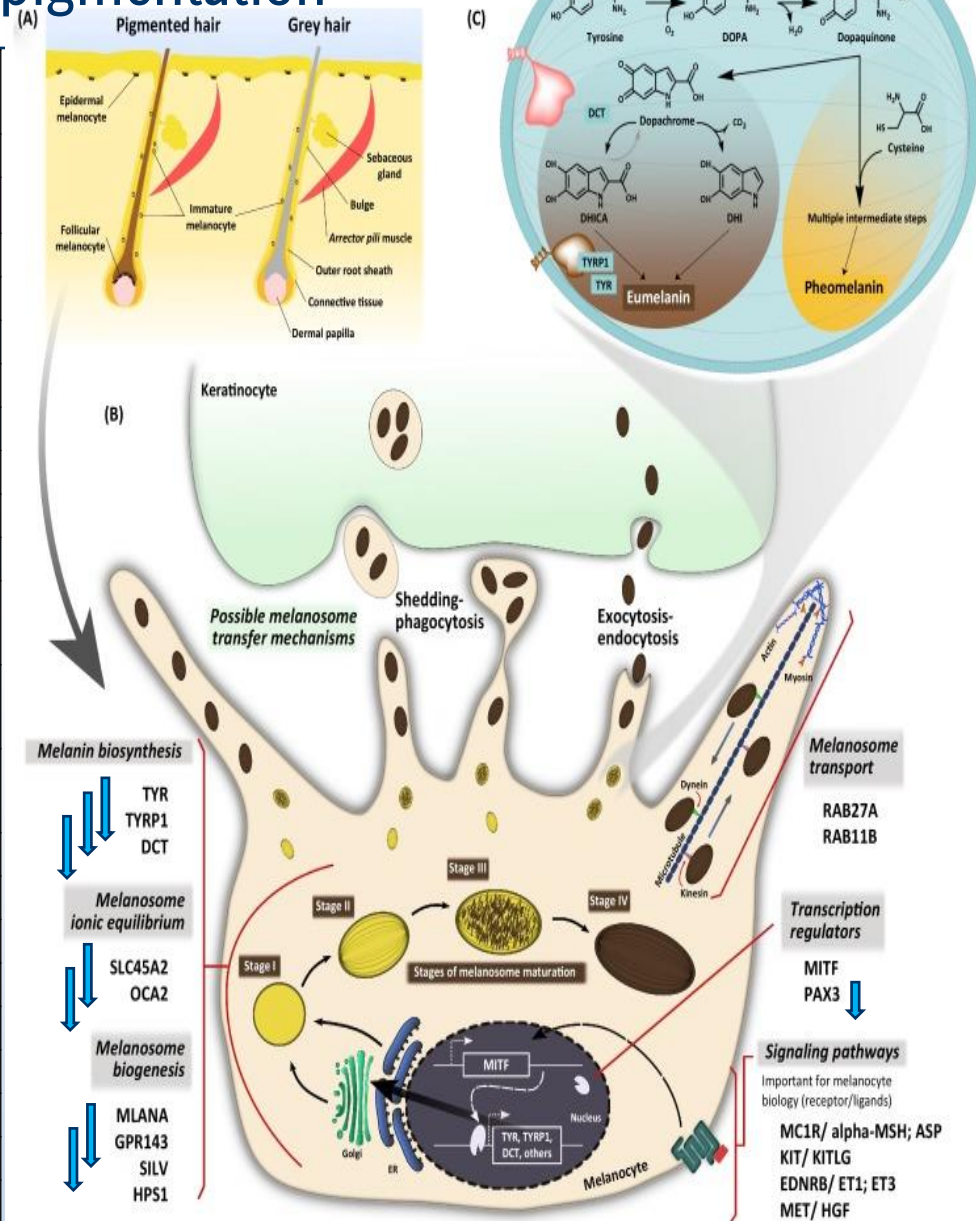


## Melanin biosynthesis



# AMPK activation: Melanocyte function in hair follicle pigmentation

Dose level: 5 mg/kg/day		Fold change			
Symbol	Function	male		female	
		Day14	Day27	Day14	Day27
DCT	Melanine biosynthesis	-1,28	-1,7	-2,51	-1,36
TYR	Melanine biosynthesis	-1,39	-1,89	-1,71	-1,43
TYRP1	Melanine biosynthesis	-1,4	-2,32	-3,24	-1,71
OCA2	Ionic equilibrium	-1,36	-2,43	-2,48	-1,48
SLC24A5	Ionic equilibrium	-1,28	-2,05	-2,77	-1,78
SLC45A2	Ionic equilibrium	1	-2,2	-1,79	-1,48
TPCN2	Ionic equilibrium	1,12	1,05	-1,04	1,07
TRPM1	Ionic equilibrium	-1,33	-2,19	-2,25	-1,71
HPS1	Melanosome biogenesis	1,53	1,42	-1,11	-1,14
MLANA	Melanosome biogenesis	-1,39	-2,04	-2,1	-1,54
PMEL	Melanosome biogenesis	-1,28	-2,14	-2,47	-1,58
RAB11B	Melanosome transport	1,17	1,11	1,02	1,01
ASIP	Signaling pathways	-1,09	-1,23	2,85	1,17
EDNRB	Signaling pathways	-1,13	-1,01	-1,03	1,22
HGF	Signaling pathways	-1,38	1,15	-1,5	-1,15
IRF4	Signaling pathways	-1,64	-1,37	-1,66	-1,95
KIT	Signaling pathways	1,12	-1,02	-1,07	1,01
KITLG	Signaling pathways	-1,17	-1,13	1,01	-1
MET	Signaling pathways	-1,1	-1,02	-1,12	1,29
MITF	Transcription regulator	-1,17	1,05	-1,25	1,05
PAX3	Transcription regulator	-1,04	-1,67	-2,29	1,17

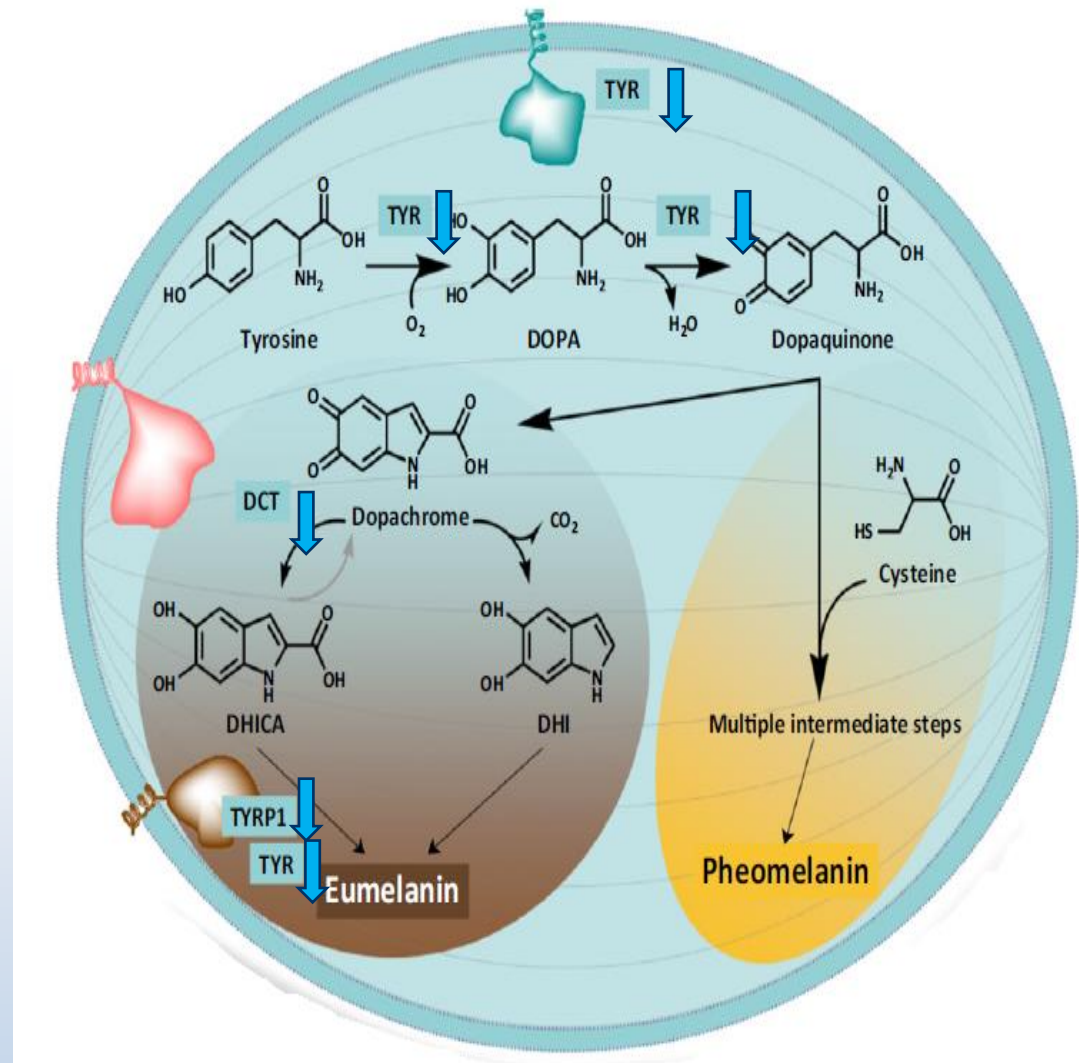


# AMPK activation:

Melanocyte function in hair follicle pigmentation: DCT↓, TYR↓, TYRP1↓

Dose level: 5 mg/kg/day		Fold change			
		male		female	
Symbol	Name	Day1 4	Day2 7	Day1 4	Day2 7
DCT	ENSCAFG0000000 5323	-1,28	-1,7	-2,51	-1,36
TYR	ENSCAFG0000000 4373	-1,39	-1,89	-1,71	-1,43
TYRP1	ENSCAFG0000000 1478	-1,4	-2,32	-3,24	-1,71

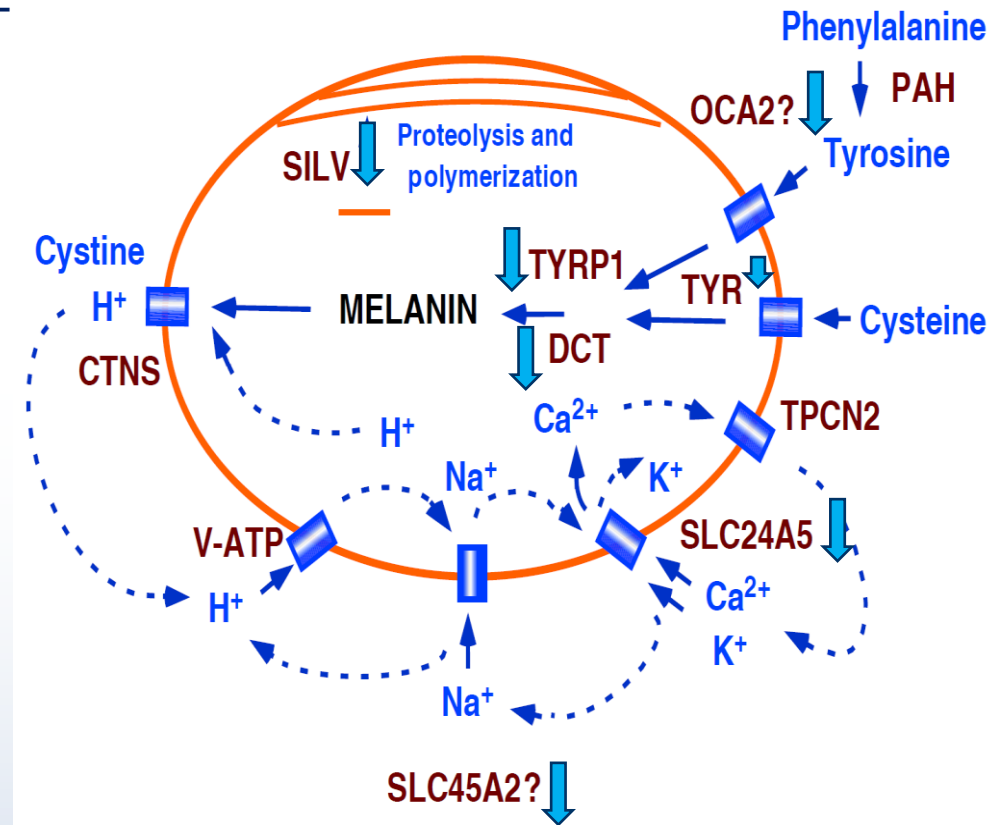
- Mutation in the TYR or TYRP1 gene results in oculocutaneous [albinism](#) (Type I and III)
- Knockout of DCT gene in mice show a diluted and lighter coat color.



# AMPK activation:

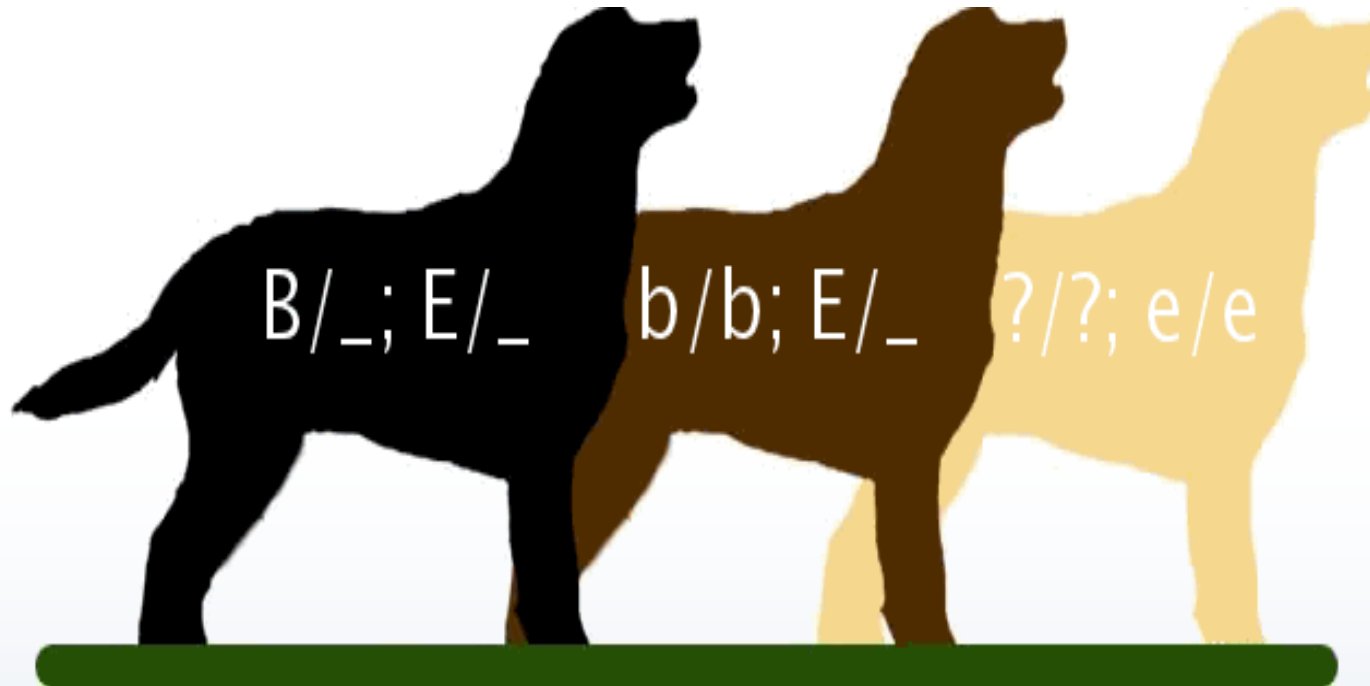
Membrane transport  $\text{SLC24A5} \downarrow$ ,  $\text{SLC45A2} \downarrow$ ,  $\text{OCA2} \downarrow$

- **SLC24A5** is a member of the potassium-dependent sodium/calcium exchanger
  - **SLC24A5** plays a role in neutralizing the pH in melanosomes (pH is critical for TYR functioning)
  - **OCA2** acts as tyrosine transporter
  - **SLC45A2** membrane transporter directing the traffic of melanosomal proteins
- Mutations in **SLC24A5** or **SLC45A2** or **OCA2** results in oculocutaneous albinism



# AMPK activation:

## Comparison with Labrador retriever coat colour genetics



Black:  $BBEE$  /  $BBEe$  /  $BbEE$  /  $BbEe$

Chocolate:  $bbEE$  /  $bbEe$

Yellow:  $Bbee$  /  $Bbee$  (CC....darkest / cc... lightest)

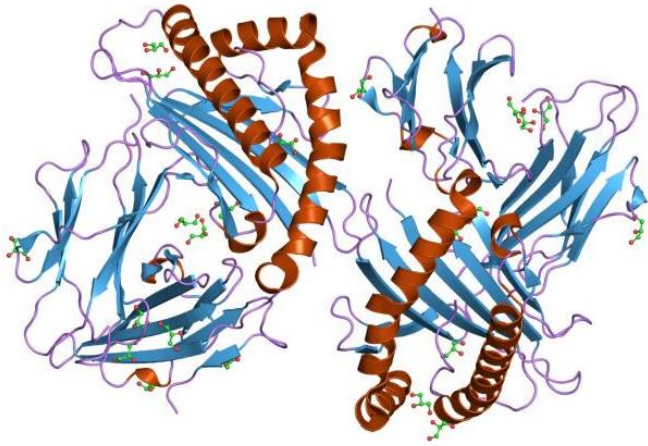
B....TYRP1

E....MC1R

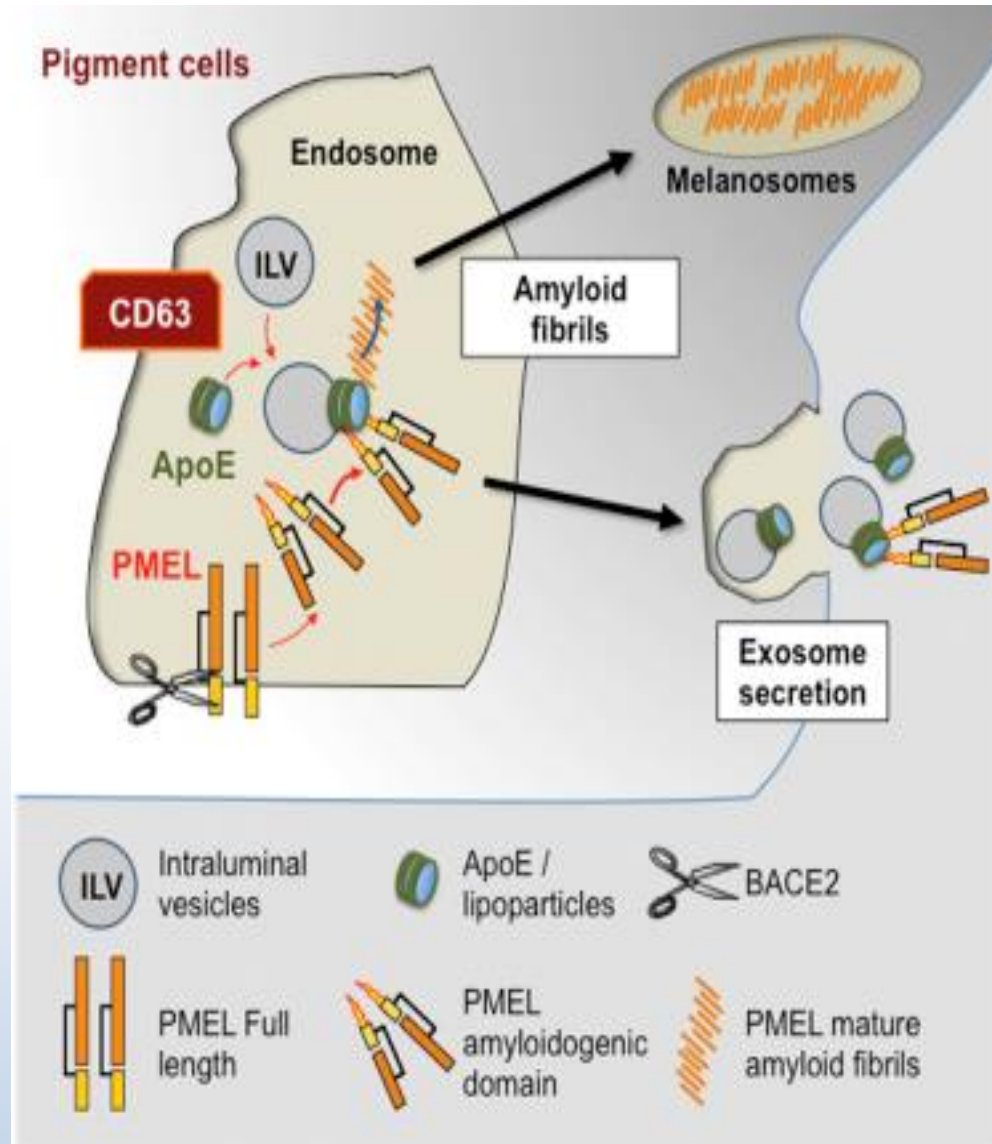
C.... SLC45A2

# AMPK activation: PMEL ↓ MLANA ↓ (melanosome biogenesis)

- PMEL (SILV) is a component of the fibrillar matrix for the polymerization of eumelanin
- MLANA (melan-A) is required for expression, trafficking and stability of PMEL



PMEL Xray structure of a dimer



# AMPK activation:

## Summary of toxicogenomic analyses

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- Decreased expression of key enzymes of **melanin biosynthesis** (DCT , TYR, TYRP1)
- Decreased expression of key genes necessary for **melanosome biogenesis** (MLANA, PMEL) and **melanosome membrane transport** (SLC45A2, SLC24A5, OCA2)
- In literature, AMPK-activators adiponectin and AICAR also down-regulated melanogenesis in an *in vitro* assay

# AMPK activation: Fur observations during recovery

Male no. 201

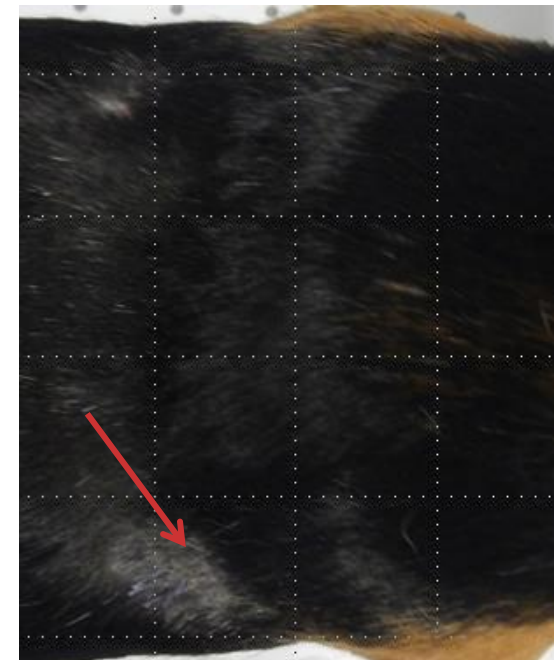
Day -6



Day 56

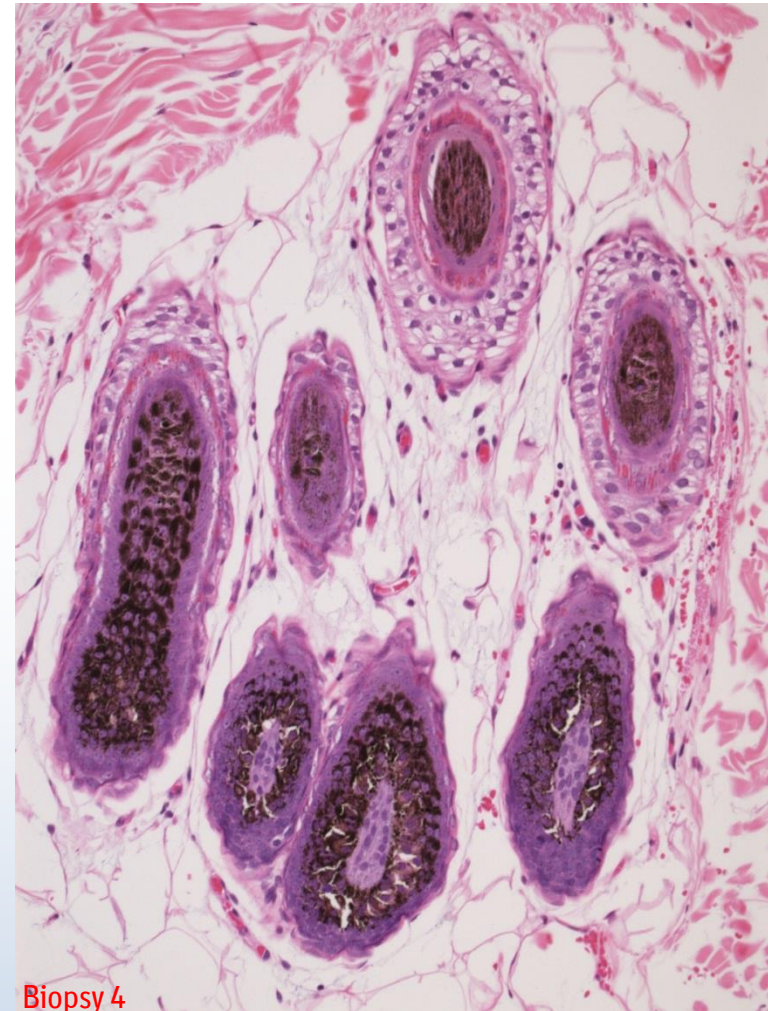
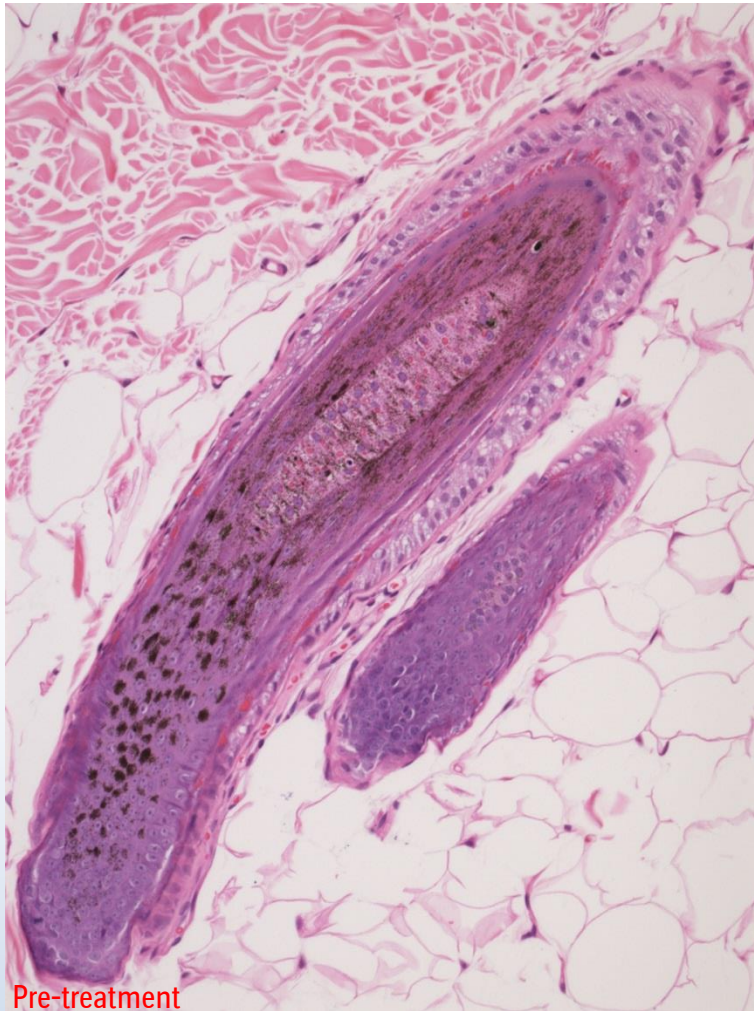


Day 63

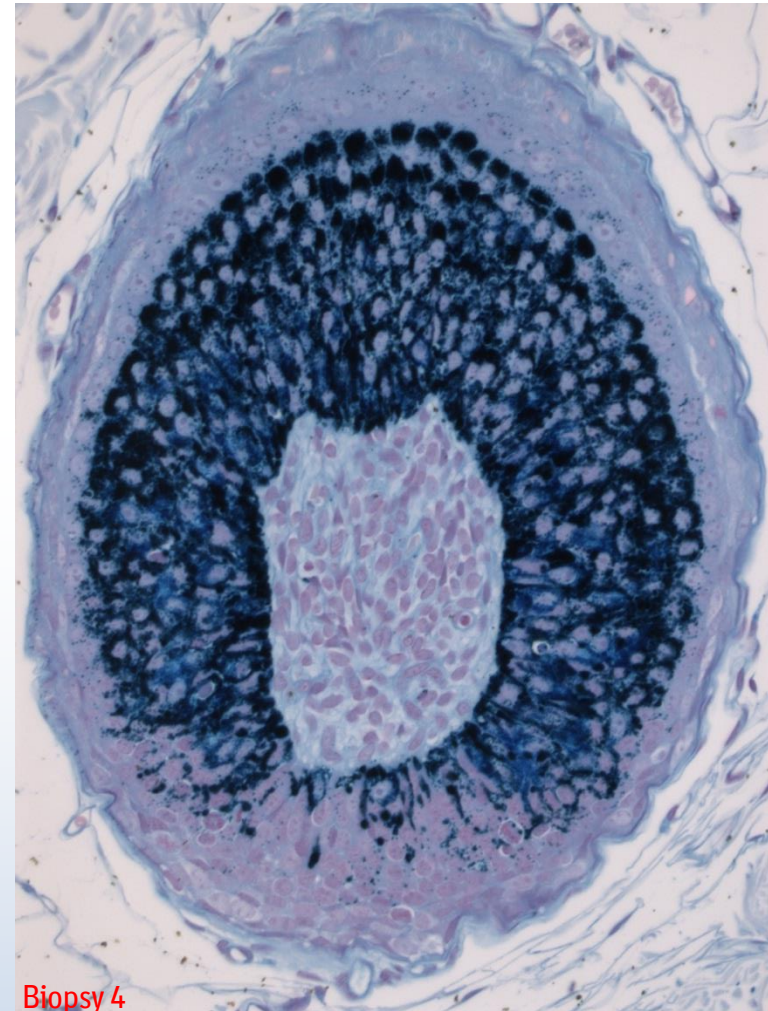
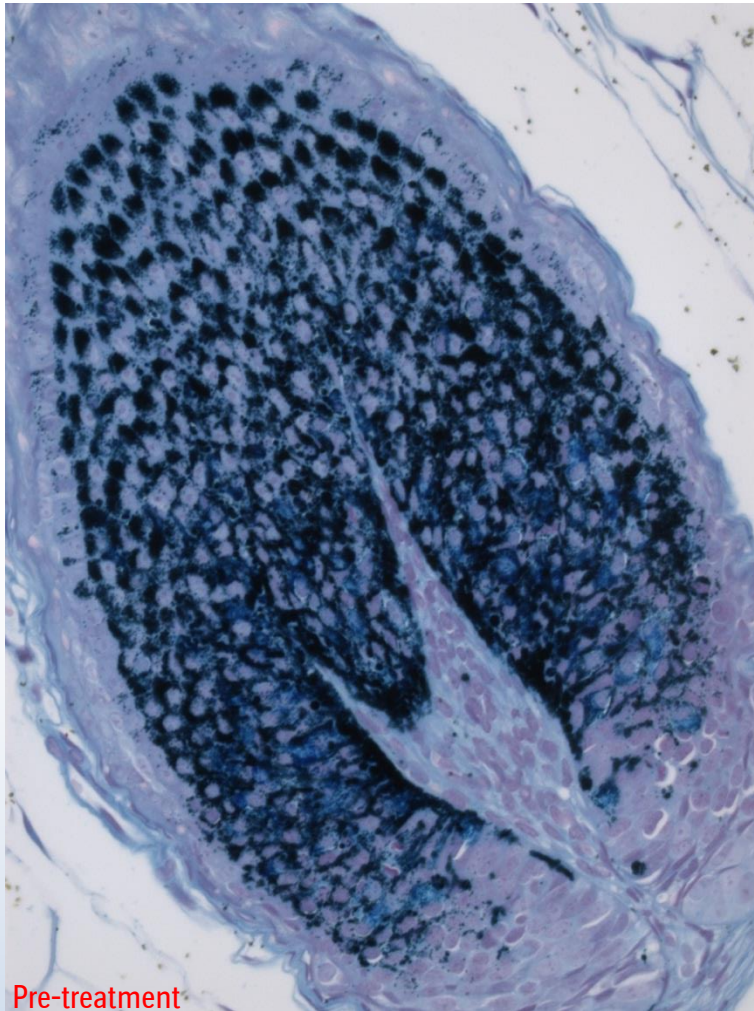


Hair discoloration in the shaved area of biopsy sampling

# AMPK activation: Histopathology - H & E



# AMPK activation: Histopathology - Schmorl



# AMPK activation: Post-scriptum

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- Why so late?
  - Hair pigmentation is active only during the anagen phase
- Why not the skin?
  - Epidermal and follicular melanocyte pigmentation are independently regulated

# AMPK activation

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Thanks for your attention!!